Does a stretch-inactivated cation channel integrate osmotic and peptidergic signals?

TO THE EDITOR— In a recent paper in *Nature Neuroscience*, Chafke and Bourque[1] assert that "mechanisms underlying osmoreception [in osmosensory neurons] are understood," and more specifically, that a stretch-inactivated cation channel (SIC) is a point of "molecular convergence" for osmotransduction and peptide-induced excitation. An enticing idea, but the data do not stand up to scrutiny.

Gd^{3+}, with its many side effects on calcium[2], potassium[3], sodium[3], non-selective[4] and cation-selective[5] channels, is a problematic diagnostic tool[6]. Nevertheless, the evidence offered for "molecular convergence" is that 10 μM Gd^{3+} and 100 μM Gd^{3+} shorten the mean open times (MOT) of peptide-stimulated and "control" SICs by similar percentages. This MOT comparison is made against previously published and highly variable findings from which "representative excerpts"[8,9] were 10–100 ms bursts, unlike the present events (Fig. 3a).

Figure 6, illustrating peptide-stimulated events modulated by 100 μM Gd^{3+}, is disconcerting for several reasons. The 100 μM Gd^{3+} produces a fast-flickery block of cation channels (including SICs in mammalian muscle[7]), yet the single-channel events illustrating Gd^{3+} block are distinctly larger than control currents. Earlier work[9] showed reduced single-channel amplitude with Gd^{3+}, and these old data constitute the controls for Fig. 6. The finding that the MOT for channel activity occurring in long bursts[8,9] is similar to the MOT of openings that seldom occur in bursts (the new data, Fig 3a) is hardly a flag that identical proteins produced both responses. Also, Gd^{3+} interacts strongly with many anions, including proteins[10], so whole-cell responses to 200 μM Gd^{3+} (Fig. 6d), which may reflect action on the peptides and/or multiple channel types, are not grounds for concluding that identical channels carry peptide-stimulated and osmotransducing currents.

Ongoing channel activity can be easily characterized as 'stretch-inactivating' by applying brief pulses of pipette suction[11], but Chafke and Bourque never use this direct approach to show peptide-activated channels are SICs. Instead, they present one dose–response curve in which the steady-state P_{open} is approximately 2-fold higher at 0 cm H_2O than at any other pressure in a range of 175 cm H_2O. The two-fold dynamic range for SIC activity is unimpressive. We are assured that "in 12 patches... P_{open} of peptide stimulated channels could be modified by changing hydrostatic pressure". This is not data—nor is the allusion to three [other] patches in which "channel P_{open} varied as a bell-shaped function of pipette pressure." Statistics are needed, as well as more current traces.

How reliable is the bell-shaped response? Previously[8,9] the authors renormalized applied pressures so that "0 cm H_2O" did not signify atmospheric pressure, but rather the pressure at which NP_{open} was maximum. Therefore, from Fig. 5b one cannot infer that membrane tension increased on either side of 0 cm H_2O (ref. 11). Furthermore, channels in cell-attached patches can experience uncontrolled variations in kinetics, so that normalizing data records to the highest activity inevitably leads to bell-shaped curves, particularly when the dynamic range is only two.

In previous papers claiming that SICs underlie osmotransduction[8,9], the reported pressures were about 50-fold smaller (±2 cm versus ±100 cm H_2O; Fig 6b). Several years ago, we alerted Dr. Bourque that the published pressures seemed extremely low—but this discrepancy goes unmentioned and certainly invalidates any comparison between the present data and the older data (Fig. 6b).

We are not convinced that "…mechanisms underlying osmoreception are understood…" and we disagree that the new data support a "molecular convergence".

Frederick Sachs[1], Catherine E. Morris[2] and Owen Hamill[3]

[1]*Physiology and Biophysical Sciences, 320 Cary Hall, SUNY, Buffalo, New York 14214, USA*

[2]*Departments of Medicine and Biology, University of Ottawa, Ottawa Hospital, 725 Parkdale Avenue, Ottawa, Ontario K1Y 4K9, Canada*

[3]*University of Texas Medical Branch, Department of Physiology and Biophysics, 301 University Blvd., Galveston, Texas 77555, USA*

email: sachs@buffalo.edu, cmorris@lri.ca or ohamill@utmb.edu

REPLY— Sachs and colleagues dispute our conclusion that stretch-inactivated cation channels serve as a point of molecular convergence for osmotic and peptidergic modulation in magnocellular neurosecretory cells (MNCs)[1]. It is argued that data reported in this paper cannot be compared with previous results from our laboratory[8,9] due to differences in channel properties and because Gd^{3+} lacks selectivity as a pharmacological probe.

They assert that traces in Fig. 6a of our report[1] suggest that Gd^{3+} increases channel current amplitude, whereas we had previously reported a small (~10%) reduction in current amplitude[9]. Unlike in the previous study[9], however, the recordings shown in Fig. 6a were obtained from different cells, not from a single patch. The excerpts shown were simply selected to illustrate the effects of Gd^{3+} on open time. Because it is unlikely that the trans-patch voltage was precisely the same in these two particular recordings, it is inappropriate to compare current amplitudes. We maintain that, at equivalent voltages, Gd^{3+} causes a reduction in current amplitude.

It is stated that channel openings shown in earlier studies[8,9] consisted of bursts of 10–100 ms, unlike the events shown in the recent paper[1]. We have acknowledged the complexity of SIC kinetics in MNCs[9]. Although we did not overtly emphasize bursting in our recent paper[1], bursts were nonetheless present and can be seen in many of the traces shown, including in Fig. 6a. More importantly, the average mean open time reported in our earlier study (1.65 ± 0.08 ms; ref. 9) is indeed consistent with the data shown in our recent paper (MOT ≈ 1.5 ms; Fig. 3a)[1].

It is also implied that interactions between Gd^{3+} and exogenously applied peptides, or other ion channels, might have caused the loss of peptide-evoked and/or resting currents in our experiments. We do not dispute that Gd^{3+} can have a variety of effects on other types of ion channels[9], and it is indeed conceivable that Gd^{3+} might have interfered with the ability of the peptides to activate receptors.