SUPPLEMENTAL MATERIAL

For Reeves *et al.* "The Sodium-calcium Exchanger is a Mechanosensitive Transporter"

1. ***Measurement of fura-2 release during superfusion.*** Cells expressing the Δ(241-680) mutant were pretreated with ATP/Tg and preincubated in 100/40 Na/K-PSS containing 1 μg/ml grancidin and 0.3 mM EGTA as described in Methods. The cells were superfused with 5 ml of 100/40 Na/K-PSS containing 0.3 mM EGTA and the superfusate was collected in 13 x 100 mm test tubes. Subsequently, total cellular fura-2 was determined by permeabilizing the cells with 0.1 mg/ml digitonin and 100/40 Na/K-PSS containing 0.3 mM EGTA. Fluorescence of each solution was measured at 380 nm excitation and 510 nm emission in a Photo Technology International RF-M 2001 fluorometer. $MnCl_2$ was added to a final concentration of 3.3 mM from a stock of 0.1 M to quench fura-2 fluorescence and the difference in readings before and after the Mn quench was taken as amount of fura-2. In some of the superfusate samples, readings taken after Mn quench were slightly higher than before, yielding negative values for released fura-2. Total fura-2 was only 15 ± 4% above background, and so precision was poor. In 4 trials, the amount of fura-2 in the superfusate ranged from -26% to +36% of the total, with a mean value (± SEM) of 1.9 ± 18%. We conclude that superfusion does not induce a large-scale release of fura-2 from the cells.

2. Imaging of calcein-loaded cells during superfusion and osmotic volume changes. The data in Fig. S-1 show fluorescent images of Δ(241-680) cells loaded with calcein and treated with gramicidin in 70/70 Na/K-PSS. The images in the upper row show the cells in 70/140 Na/mannitol PSS (green-control), and after changing the

medium to 70/500 Na/mannitol (red-hypertonic). Shrinkage of the cells in the hypertonic medium is obvious by inspection of the two images; the merged images show yellow where the red and green overlap, with green edging depicting the larger, original cell volume. The complementary experiment conducted with a hypotonic medium change (70 Na-PSS) is depicted in the second row. Swelling of the cells in the hypotonic medium is again evident by inspection and the merged images show yellow cells edged with red. The bottom row of images shows cells in 70/70 Na/K-PSS + 0.3 mM EGTA (control) and during the application of 15 ml of the same medium (Iso Applied – red). No volume change occurred in this case: the merged images show a modest displacement of some of the cells, with red edges on one side balanced by green edges on the opposite side. These small displacements may contribute to the decline in fluorescence intensity within the regions of interest that we monitor during fluorescence measurements (see Methods). A partial, but variable, loss of focus during solution flow also occurs, and this would likewise contribute to the reduction in fura-2 fluorescence intensities. Our evidence, however, suggests that no dramatic changes in cell volume or shape occur during solution superfusions.

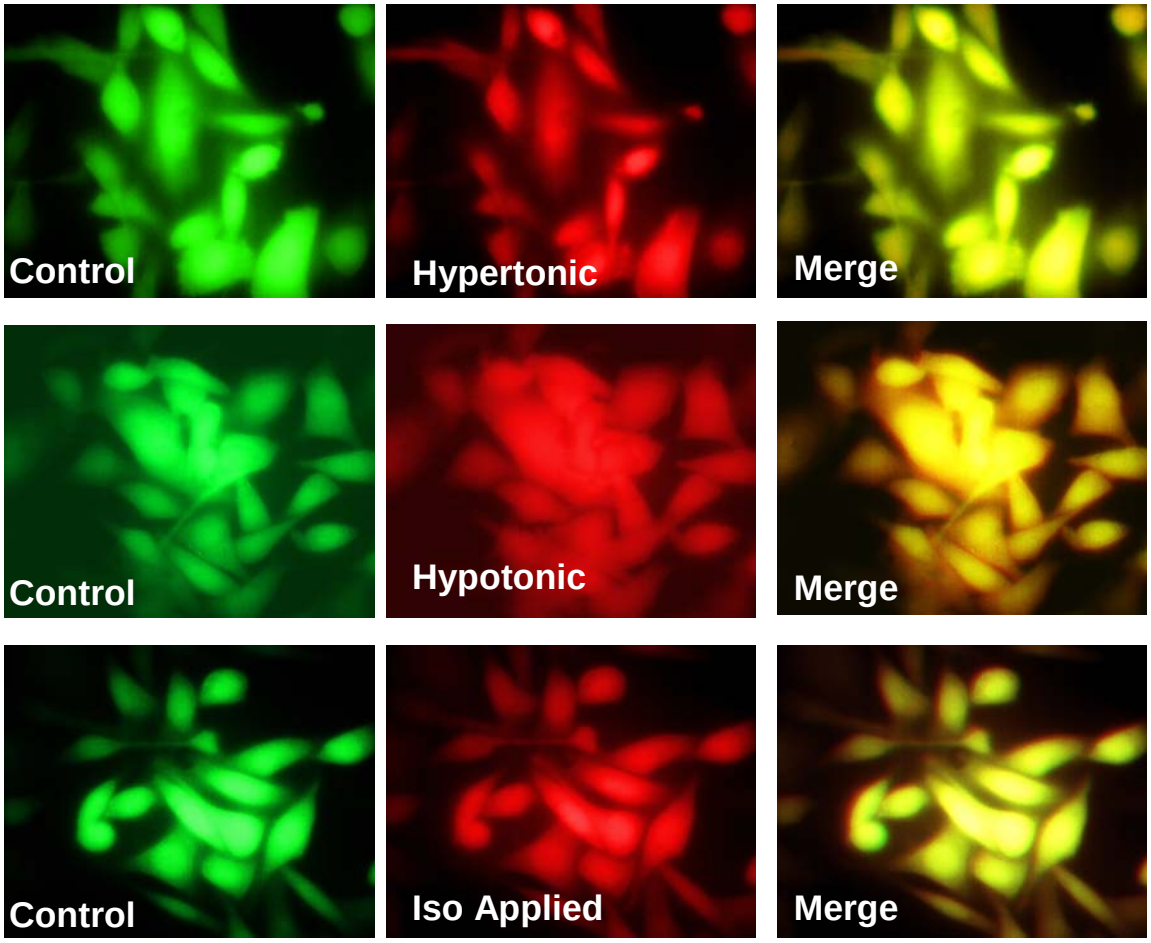
3. ***Ba^{2+} influx during osmotically-induced volume changes.*** For the experiment shown in Fig. S-2, Panel A, Δ(241-680) cells were equilibrated in 70/70 Na/K-PSS and, 2 min prior to recording, the medium was changed to 70/140 Na/mannitol-PSS. Exchange activity was then initiated by superfusing 5 ml 2 mM Ba^{2+} in either an isotonic medium (70/140 Na/mannitol-PSS), a hypotonic medium (70 Na-PSS) or a hypertonic medium (70/500 Na/mannitol-PSS). Hypotonic conditions stimulated Ba^{2+} uptake. Hypertonic conditions attenuated the amplitude of the rapid phase and strongly inhibited Ba^{2+} uptake

during the slow phase. At the second arrow (hypertonic trace only), 5 ml of hypertonic Ba^{2+} solution was reapplied, and this induced a second period of rapid Ba^{2+} influx. The data in Panel B show the results of essentially identical experiments carried out with non-transfected CHO T cells. In this case there was no difference in Ba^{2+} uptake between isotonic and hypertonic media, and only a slight stimulation in the hypotonic medium. (The second superfusion of hypertonic Ba^{2+} solution was not done in these experiments). For the non-transfected CHO T cells, Ba^{2+} uptake is probably due to store-operated channel activity. The levels of Ba^{2+} uptake in these experiments are higher than those shown in Fig. 2, Panel B, trace c. This may reflect stimulation of store-operated Ba^{2+} entry by a combination of the reduced ionic strength in these experiments, as well as the expected negative membrane potential due to the reduction in the external cation concentration.

The rates of Ba^{2+} influx were computed as the slopes of the traces between 35-45 s for the rapid phase and 120-150 s for the slow phase and are shown in panels C and D for the Δ(241-680) cells. Panel E shows the rates of Ba^{2+} influx measured between 120-150 s for the non-transfected CHO cells. The rapid phase rates (Panel C) for the hypo- and hypertonic conditions were not significantly different from the isotonic control rates (t-test), although significance was attained ($p \sim 0.004$) if the hypo- and hypertonic rates were compared. Analysis of variance showed a significant difference between groups ($F = 8.36$, $p = 0.006$). For the slow phase rates (Panel D), both the hypo- and hypertonic conditions were significantly different from the isotonic controls by the t-test ($p < 0.05$). Note the difference in scale between panels C and D; the rapid phase rates are about 10 times greater than the rates for the corresponding slow phases. Hypotonicity stimulated

the rate of slow-phase Ba^{2+} uptake by 67% and hypertonicity inhibited by 44%. For the non-transfected CHO T cells, the rate of Ba^{2+} influx was stimulated by 50% in the hypotonic medium ($p = 0.047$).

We conclude that hypotonicity stimulated NCX-mediated Ba^{2+} influx and hypertonicity inhibited it. The effects of the osmotic changes on the rates of Ba^{2+} uptake were relatively small in comparison to those seen with superfusion. It appears that the primary effect of hypotonicity may be to extend the duration of the rapid phase (or to slow the transition to the slow phase), since elevated rates of Ba^{2+} influx continue well beyond the period of superfusion in the hypotonic medium (30-50 s). Conversely, hypertonicity may accelerate the transition to the slow phase.


Control
Hypertonic
Merge
Control
Hypotonic
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Control
Iso Applied
Merge

Figure S-1

Figure S-1. Effects of application of isotonic, hypotonic or hypertonic solutions on cells loaded with calcein. Cells expressing Δ(241-680) were loaded for 30 min in Na-PSS + 1 mM Ca^{2+} with 3 μM calcein-AM. Gramicidin-treated cells were equilibrated in 70/70 Na/K-PSS containing 0.3 mM EGTA. For the images in the first two rows (from above), the medium was changed to PSS containing 70 mM Na + 140 mM mannitol and images were taken by fluorescence microscopy before (control-green) and after application of PSS containing 70 mM Na^+ + 500 mM mannitol (hypertonic-red) or 70 mM Na^+ (hypotonic-red). For cells in the bottom row, images were taken in 70/70 Na/K-PSS + 0.3 mM EGTA before (control) and during (Iso Applied - red) the application of 15 ml of the 70/70 Na/K-PSS medium.

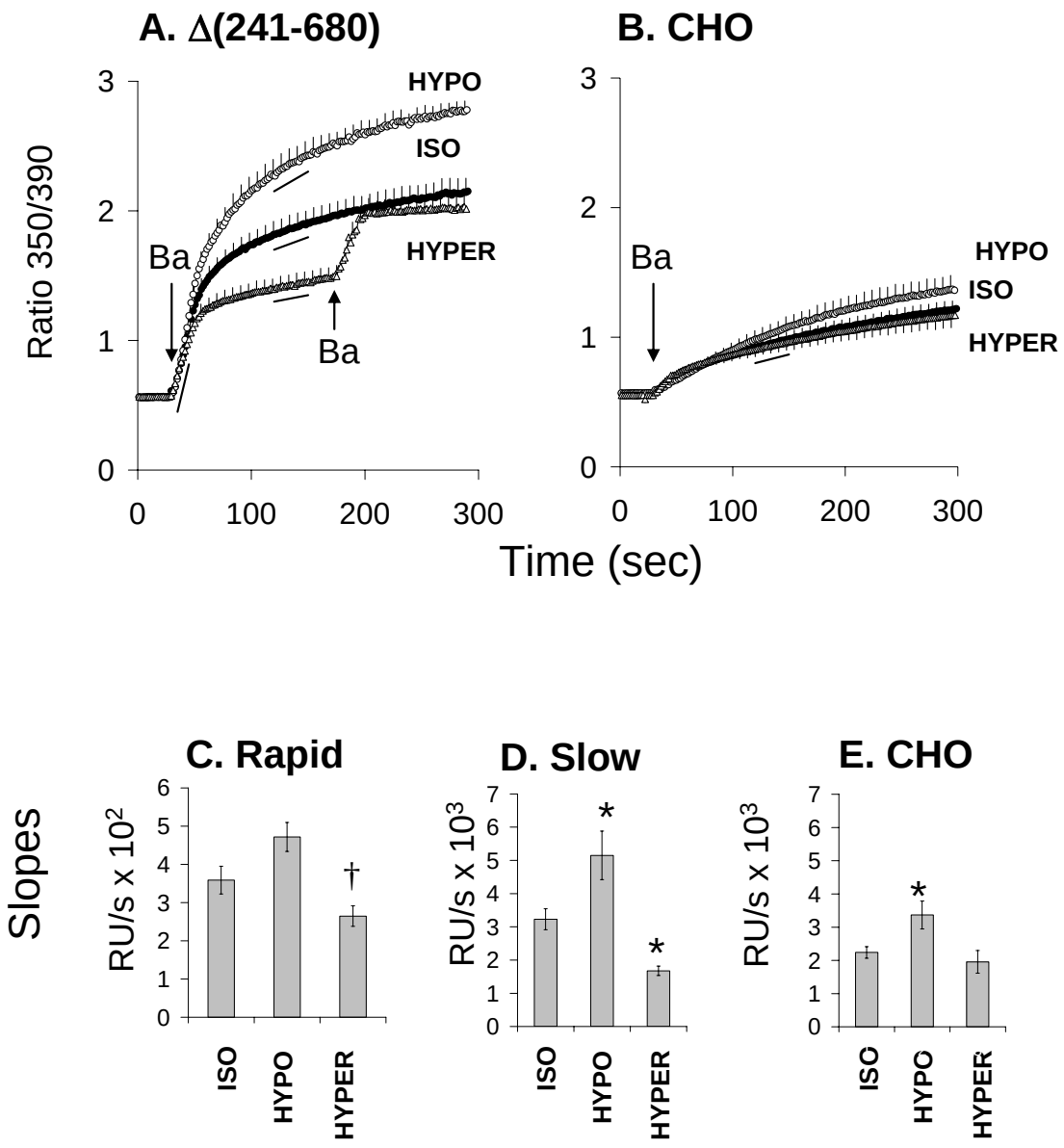
A. Δ(241-680)
B. CHO
Ratio 350/390
HYPO
ISO
HYPER
Ba
Ba
Time (sec)
Slopes
C. Rapid
D. Slow
E. CHO
RU/s x 10²
RU/s x 10³
RU/s x 10³
ISO
HYPO
HYPER
*
†

Figure S2

Figure S-2. Effect of osmolarity on Ba^{2+} uptake. A. Gramicidin-treated cells expressing Δ(241-680) were equilibrated with 70/70 Na/K-PSS containing 0.3 mM EGTA. Two min prior to beginning recording, the medium was changed to 70/140 Na/mannitol-PSS + 0.3 mM EGTA. Ba^{2+} uptake was initiated by applying 5 ml of 2 mM Ba^{2+} in 70/140 Na/mannitol PSS (Iso, n = 5), 70 Na-PSS (Hypo, n = 5) or 70/500 Na/mannitol PSS (Hyper, n = 4); as usual, the assay solutions also containing 0.1 mM EGTA to chelate residual Ca^{2+}. For the latter trace, 2 mM Ba^{2+} in 70/500 Na/mannitol PSS was applied a second time where indicated. Traces are the mean values +SEM bars for the number of cover slips given above; error bars are shown for every fourth data point. B. Results of an identical experiment carried out with non-transfected CHO T cells (n = 4 for each trace). C. Rates of Ba^{2+} uptake during the rapid phase are given as the average slopes of the individual traces over the interval of 35-45 s. D. Rates of Ba^{2+} uptake during the slow phase are given as the average slopes of the individual traces over the interval of 120-150 s. Intervals over which rates were measured are indicated by bars beneath the traces. Note that the ordinate scale in C is 10 times greater than that in D. E. Rates of Ba^{2+} uptake for non-transfected CHO T cells between 120-150 s. †, $p < 0.004$ for hypertonic vs hypotonic rates; *, $p < 0.05$ for hypotonic or hypertonic rates vs isotonic rate in panel D or for hypotonic vs isotonic in Panel E.