SUPPLEMENTARY MATERIALS

Supplementary Fig. 1 legend:

Fig. S1. Mathematical modeling of umbrella cell electrophysiological parameters. (A) Predicted changes in transepithelial conductance (TEC), V_a, TEV, and I_{sc} upon increased apical conductance of Na^+ or/and K^+ ions. (B) Predicted changes upon increased basolateral conductance of Na^+ or/and K^+ ions. (C) Predicted changes in TEV and I_{sc} upon sequentially increasing apical and then basolateral conductance of Na^+ and K^+ ions using two values for E_{Na} and E_K. (D) Circuit diagram of membranes and ion transport pathways in the umbrella cell.

Supplementary Fig. 1 discussion:

The apical and basolateral membrane contributions to the observed electrophysiological properties can be modeled mathematically

Our data indicate that the phase 1 response of outward bowing and subsequent reversal of the electrophysiological parameters during phase 2 may result from the successive stretch of the apical and then basolateral membranes of the umbrella cell layer. To further confirm this possibility and also gain insight into the molecular mechanisms involved, we modeled umbrella cell electrophysiological responses assuming that the apical and basolateral membranes are stretched sequentially, and Na^+ and K^+ permeable ion channels are modulated upon stretch.

We and others have previously reported that stretch stimulates Na^+ absorption via the amiloride-sensitive ENaC in the apical membranes of the umbrella cell layer (Lewis *et al.*, 1977; Lewis and Wills, 1983; Lewis and Hanrahan, 1985; Wang *et al.*, 2003). Therefore, we first modeled the impact that an increase in the apical membrane conductance of Na^+ would have on several electrophysiological parameters. In addition to causing apical membrane depolarization (V_a), the increase in Na^+ absorption would result in TEV hyperpolarization, an increase in I_{sc}, and an increase in transepithelial conductance (Fig. S1A), a response identical to that observed during phase 1 of outward bowing. We also modeled the condition where the apical membrane conductance was limited to an increase in K^+ conductance via K^+-permeable ion channels. A response largely opposite of that observed for increased Na^+ conductance was predicted (Fig. S1A). If the increase in apical membrane conductance was contributed equally by both Na^+ and K^+ permeable ion channels, a response similar to that observed for opening of Na^+ permeable ion channels was observed (Fig. S1A), indicating that Na^+ permeable ion channels may play a major role in the response observed when the apical membrane is stretched.

We also modeled events at the basolateral membrane, and observed that absorption of just Na^+ permeable ion channels resulted in basolateral membrane and TEV depolarization, increased transepithelial conductance, and a decrease in the magnitude of I_{sc} (Fig. S1B), a response similar to that observed during phase 2 of outward bowing. Likewise, if stretch of the basolateral membrane resulted in an equal increase in conductance through both Na^+ and K^+ permeable channels, we would observe an increase in transepithelial conductance and a decrease in TEV and I_{sc}. In contrast, opening of K^+ channels alone generally gave an opposite response to that we observed (Fig. S1B). Taken together these data indicated that the second phase of outward bowing may occur in response to closing of K^+ channels or increasing the influx of Na^+ ions. Finally, we modeled the situation where we increased conductance of equal amounts of Na^+ and K^+ ions at the apical membrane, followed by increasing conductance of Na^+ and K^+ ions at the basolateral surface. We used different values for E_{Na} (45 or 55 mV) and E_K (-75 or -105mV), but in either case modeling showed an initial phase marked by hyperpolarization of TEV, a rise in I_{sc} and transepithelial conductance, followed by a second phase characterized by depolarization of TEV and a fall in I_{sc} (Fig. S1C). In summary, mathematical modeling provides further support of the distinctive function of the apical and basolateral membranes in modulating the electrophysiological responses to umbrella cell stretch.

Supplementary methods:

Mathematical modeling of electrophysiological parameters

Our mathematical model was based on previously described electrophysiological analysis of umbrella cells (Lewis *et al.*, 1977; Lewis *et al.*, 1978; Lewis and Wills, 1983; Lewis and Hanrahan, 1985; Horisberger, 1991) and the circuit diagram shown in Fig. S1D. The TER and TEV can be estimated from the following equations: TER = $(R_a+R_b)*R_j/(R_a+R_b+R_j)$ and TEV = $(V_a+V_b)*R_j/(R_a+R_b+R_j)$, where R_j = the tight junction resistance, V_a = the apical membrane voltage, and V_b = the basolateral membrane voltage. Because $Rj \cong \infty$ (Lewis *et al.*, 1977; Lewis and Wills, 1983; Lewis and Hanrahan, 1985), and remains very high even during stretch (Wang *et al.*, 2003), the above equations can be simplified to TER = R_a+R_b and TEV = V_a+V_b. The transepithelial conductance is equal to 1/TER. Estimates of R_a (20000 Ω•μF), R_b (1000 Ω•μF), V_a (40 mV) and V_b (- 70 mV) were described previously (Lewis *et al.*, 1977; Lewis *et al.*, 1978; Lewis and Wills, 1983; Lewis and Hanrahan, 1985; Horisberger, 1991). V_a and V_b are essentially dependent on the potential difference of Na^+ and K^+ and their relative conductances according to the following equation: $V_{a/b} = (G_{a/bNa}*E_{Na} + G_{a/bK}*E_K)/(G_{a/bNa} + G_{a/bK})$, where $G_{a/bNa}$ and $G_{a/bK}$ are the respective conductances of Na^+ or K^+ at the apical (a) or basolateral (b) membrane domain. The equilibrium potential for Na^+ (E_{Na}) or K^+(E_K) was determined using the Nernst equation and typical values of E_{Na} (45 – 55 mV) and E_K (-75 – -105 mV). Isc was calculated by $(V_a + V_b)/ (R_a + R_b)$.

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