Generally Physiological

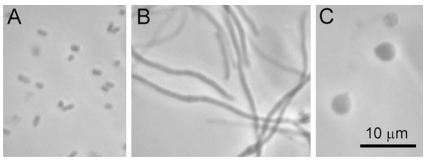
Bacteria under pressure, calcium channel internalization, and why cockroaches avoid glucose-baited traps

The Journal of General Physiology

This month's installment of *Generally Physiological* explores how the bacterium that causes cholera responds to pressure, a mechanism for the Ca^{2+}-dependent internalization of L-type calcium channels, and how cockroaches learned to avoid sugar-baited traps.

Defining the response to pressure

Bacteria, which are frequently exposed to rapid shifts in osmotic gradient, can survive potentially lytic increases in hydrostatic pressure through the emergency activation of mechanosensitive channels that mediate osmolyte release. In this issue, Rowe et al. created giant spheroplasts to perform the first patch-clamp analysis of tension-activated currents in the plasma membrane of *Vibrio cholera* (strains of which cause cholera). *V. cholera* tolerates environments of widely varying osmolarity—ranging from the intestinal lumen, to fresh water, to estuaries, to the sea. Rowe et al. (2013) found that exposure to saturating pressure ramps elicited a two-wave response, similar to that observed in *Escherichia coli*, with small-conductance MscS-like channels activating at a lower pressure than did large-conductance MscL-like channels. The gating and conductive properties of *V. cholerae* channels were comparable to those of their *E. coli* counterparts, and channel activation was shifted toward higher tensions in the presence of trehalose (an osmolyte that is accumulated under hypertonic conditions). A comparison of the ensemble responses of channels in the two species indicated that, whereas MscS-like channels were less dense in *V. cholerae* than in *E. coli*, MscL-type channels were present at higher density, with a higher overall mechanosensitive channel density in *V. cholerae*. Surprisingly, however, *V. cholerae* was more sensitive than *E. coli* to abrupt decreases in osmolarity, leading the authors to postulate that the increased number of MscL channels might help compensate for other traits rendering *V. cholerae* vulnerable to osmotic shock.

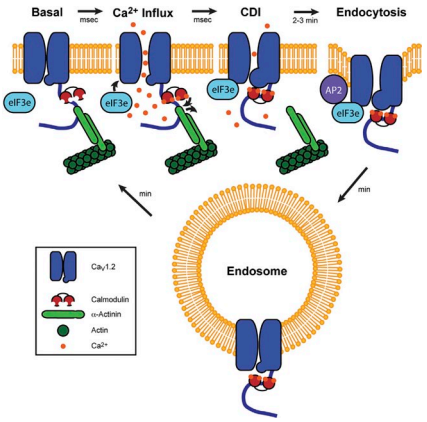


***V. cholerae* (A) incubated in cephalexin, which blocks septation, form long filaments (B); subsequent incubation with EDTA and lysozyme elicits spheroblast formation (C). (From Rowe et al., 2013.)**

Bacteria under pressure, calcium channel internalization, and how roaches learned to avoid sugar-baited traps

Competing for $Ca_v1.2$

L-type Ca^{2+} channels provide a major route of Ca^{2+} entry, thereby mediating various crucial functions in neurons and cardiomyocytes. Noting that disruption of the actin cytoskeleton decreases the activity of L-type channels, Hall et al. (2013) combined coimmunoprecipitation and immunofluorescence analysis to determine that the actin-binding protein α-actinin bound to $\alpha_1 1.2$ (the pore-forming subunit of $Ca_v1.2$, the main L-type channel in the brain). Pulldown analyses indicated that α-actinin bound to a membrane-targeting and calmodulin-binding region of the $\alpha_1 1.2$ C terminus; moreover, coexpression of dominant-negative α-actinin fragments decreased both Ba^{2+} current through $Ca_v1.2$ and $Ca_v1.2$ cell surface abundance in HEK293 cells, as did α-actinin knockdown. Calmodulin disrupted α-actinin binding to $\alpha_1 1.2$ in the presence—but not absence—of Ca^{2+}, and Ca^{2+} inhibited coimmunoprecipitation of α-actinin and $\alpha_1 1.2$ from brain extracts, suggesting that Ca^{2+}-bound calmodulin competes with α-actinin for binding to $\alpha_1 1.2$. Prolonged depolarization can lead to a decrease in the cell surface abundance of L-type channels; these data suggest that this may involve Ca^{2+} entry and the subsequent displacement of $\alpha_1 1.2$ from α-actinin by Ca^{2+}-calmodulin. Indeed, exposure to the Ca^{2+} ionophore ionomycin or BayK8644 (which increases Ca^{2+} influx through

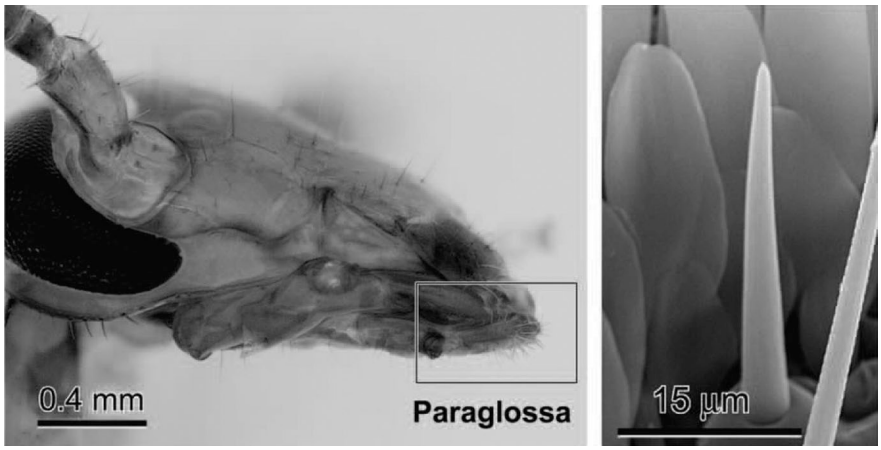


Model for calcium-dependent endocytosis of $Ca_v1.2$. (Reprinted from *Neuron*, Volume 78, Issue 3, 483–497, Hall et al. Copyright 2013, with permission from Elsevier.)

L-channels) decreased surface abundance of $Ca_v1.2$ in HEK293 cells and cortical cultures, as did prolonged depolarization of hippocampal neurons. Ionomycin, FPL64176 (which also increases Ca^{2+} influx through L-channels), or glutamate decreased coimmunoprecipitation of α-actinin and $Ca_v1.2$ from forebrain slices; glutamate's ability to do so was blocked by pharmacological antagonism of L-type channels but not of the NMDA receptor. Whole-cell patch recordings of cultured hippocampal neurons revealed rundown of L-type currents with 100-ms depolarizations at 0.2 Hz; rundown was diminished by expression of a calmodulin mutant deficient in calcium binding or by an inhibitor of endocytosis. The authors thus propose that calcium influx triggers L-channel endocytosis through a mechanism that involves disruption by Ca^{2+}-calmodulin of α-actinin binding to $\alpha_1 1.2$.

A change of taste

Cockroaches, which have been around for a very long time, have a remarkable ability to adapt to environmental changes. For instance, the German cockroach (*Blatella germanica*) adapted to the use of baits combining glucose with insecticide by developing a heritable aversion to glucose. Noting that, in insects, gustatory receptor neurons (GRNs) are modal-specific and drive appropriate behaviors (so that substances activating sugar GRNs elicit appetitive behavior, whereas those that activate bitter GRNs elicit aversive behaviors), Wada-Katsumata et al. (2013) determined that glucose-sensitive sensilla on the mouthparts of German cockroaches contained four distinct GRNs (GRN1–4). In wild-type cockroaches, the sugars fructose and glucose stimulated GRN1, but not GRN2, whereas caffeine (a bitter substance) stimulated GRN2 (but not GRN1). Further analysis revealed that various substances that stimulated feeding activated GRN1, whereas aversive substances stimulated GRN2. In glucose-averse cockroaches, however, glucose (and other substances attractive to wild-type cockroaches but aversive to glucose-averse cockroaches, such as methyl α-D-glucoside) stimulated GRN2 as well as GRN1, whereas fructose, which remained attractive, failed to stimulate GRN2. Glucose activation of GRN1 was greater in wild-type cockroaches than in glucose-averse roaches. Moreover, whereas combining glucose and fructose increased both the feeding response and GRN1 firing compared with those elicited by fructose alone in wild-type cockroaches, glucose suppressed responses to fructose in glucose-averse cockroaches. Thus, the authors conclude that the development of glucose-aversive behavior depends on acquisition of sensitivity to glucose by bitter GRNs, enabling the rapidly evolving cockroach to survive human attempts at eradication.



Side view of cockroach paraglossa, with a closeup of a taste sensillum used in electrophysiological recording. (From A. Wada-Katsumata et al. 2013. *Science*. 340:972–974. Reprinted with permission from AAAS.)

Elizabeth M. Adler
Executive Editor, *JGP*
eadler@rockefeller.edu

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