Generally Physiological

Of capturing fish, mobilizing enzymes, and a surprising source for serotonin

This month's installment of *Generally Physiological* considers a nonconventional mechanism of serotonin release in the dorsal raphe nucleus, the use of insulin by fish-hunting cone snails to induce hypoglycemic shock in their prey, and how the heat of catalysis might enhance enzyme diffusion.

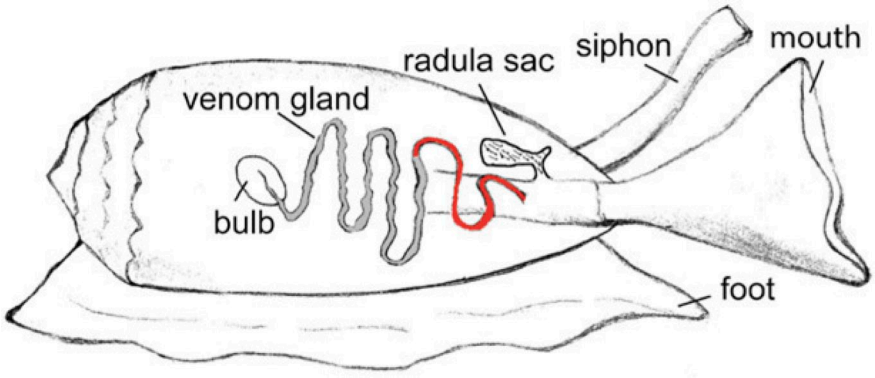
A surprising source for serotonin, a shocking use for insulin, and how the heat of catalysis might enhance enzyme diffusion

A surprising source for serotonin

Extracellular serotonin acts through somatodendritic 5-HT1A autoreceptors to suppress the activity of serotonergic neurons of the dorsal raphe nucleus through an autoinhibitory negative feedback mechanism. The concentration of this pool of extracellular serotonin increases when its reuptake is inhibited by the well-known class of antidepressants known as selective serotonin reuptake inhibitors, suggesting that there is ongoing release of serotonin into the extracellular space. Using 5-HT1A autoreceptor-activated GIRK currents of patched serotonergic neurons to assay extracellular serotonin in rat brain slices, and suppression of firing rate to evaluate its autoinhibitory activity, in this issue Mlinar et al. now show, that, remarkably, autoinhibitory serotonin release appears to occur through a nonexocytotic mechanism. Release persisted despite inhibition of serotonin uptake into synaptic vesicles (a manipulation that suppressed serotonergic inhibitory postsynaptic potentials), calcium influx, neuronal firing, or plasma membrane transporters (to rule out efflux through reverse transport). Mlinar et al. (2015) thus conclude that the release of serotonin mediating autoinhibition occurs from a nonvesicular pool through a nonexocytotic mechanism and propose that it arises instead from simple diffusion across the plasma membrane.

A shocking use for insulin

The predatory marine cone snails are known for the astonishing diversity of their venom peptides, with the venom of individual *Conus* species containing a complex mixture of numerous peptides, and different species producing venom of distinct composition. Most of the known conopeptides are neurotoxins that specifically target channels, receptor, or transporters, proving almost as useful to neuroscientists as to cone snails. In a marked departure from this scheme, however, Safavi-Hemami et al. (2015) identified specialized forms of the peptide hormone insulin in the venom of two fish-hunting cone snails, *Conus geographus* and *Conus tulipa*. Analysis of the *C. geographus* venom gland transcriptome revealed two insulin-like transcripts (Con-Ins G1 and Con-Ins G2), with the predicted mature Con-Ins G1 peptide resembling vertebrate—specifically fish—insulin. RT-PCR analysis confirmed the presence in the venom gland of insulin-like transcripts, and MS analysis showed that Con-Ins G1 and variant peptides were abundant in *C. geographus* venom. *C. geographus* uses a "net-hunting" strategy to capture fish, engulfing them in a false mouth before paralyzing them, and the venom gland of a closely related net-hunting snail, *C. tulipa*, also expressed

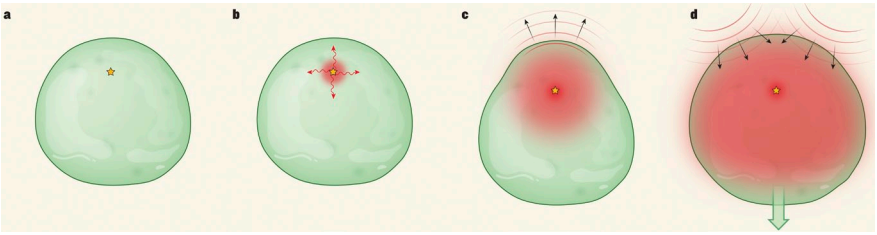


Schematic of *C. geographus*, showing the venom gland. The Con-Ins G1 transcript is most abundant in the region of the venom gland highlighted in red, as are the transcripts encoding a group of neurotoxins known as the "nirvana cabal" thought to suppress sensory circuitry in fish (Reprinted by permission from Specialized insulin is used for chemical warfare by fish-hunting cone snails. Safavi-Hemami, H., J. Gajewiak, S. Karanth, S.D. Robinson, B. Ueberheide, A.D. Douglass, A. Schlegel, J.S. Imperial, M. Watkins, P.K. Bandyopadhyay, M. Yandell, Q. Li, A.W. Purcell, R.S. Norton, L. Ellgaard, and B.M. Olivera, *Proc. Natl. Acad. Sci. USA*. 2015. http://dx.doi.org/10.1073/pnas.1423857112)

"fish-type" insulins. In contrast, insulin transcripts were absent from the venom glands of *Conus* species that use a harpoon-type strategy to capture prey, and analyses of the venom glands of mollusk and worm-hunting snails revealed expression of only molluscan-type insulins. Synthetic Con-Ins G1 lowered blood glucose concentration when injected into zebrafish, and rendered them hypoactive when applied to the surrounding water. The authors thus propose that fish-hunting cone snails that use a net-hunting strategy release insulin to induce hypoglycemic shock in prey and thereby facilitate their capture.

Mobilizing enzymes

The diffusion of various enzymes is enhanced during catalysis; the mechanism underlying this substrate-dependent effect, however, has remained unclear (see Wand, 2015). Riedel et al. (2015) now propose that this enhanced diffusion is secondary to heat produced during catalysis. Single-molecule fluorescence correlation spectroscopy of catalase, urease, and alkaline phosphatase, all of which catalyze strongly exothermic reactions, revealed a linear relationship between the increase in diffusion coefficient and reaction rate (and consequently the heat produced).



Heat released at the enzyme's catalytic site (star) causes rapid asymmetric expansion of the protein (orange wave), initiating acoustic waves in the surrounding fluid that reflect back on the enzyme to enhance its diffusion. (Reprinted by permission from Macmillan Publishers, Ltd. A.J. Wand. *Nature*. http://dx.doi.org/10.1038/nature14079, copyright 2015.)

In contrast, the addition of substrate to triose phosphate isomerase, which produces much less heat during catalysis, failed to enhance its diffusion. Moreover, directly heating the heme group at the catalase active site with a laser produced a similar increase in its diffusion. The authors thus propose that, for enzymes in which the catalytic site is not located at the center of mass, the heat produced during catalysis gives rise to an asymmetric expansion of the protein, leading to differential stress at the protein–solvent interface and thereby causing it to move.

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