Molecules in motion: imaging peptides, their receptors and diffusion models

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Although neuropeptides and the G-protein coupled receptors (GPCRs) through which they operate have been well studied for more than 30 years now, many aspects of their function remain mysterious. When considering the roles of neuropeptides as transmitters in peripheral autonomic and sensory pathways, two questions remain largely unanswered: (1) can neuropeptides mediate non-synaptic neurotransmission? (2) how do neuropeptide signalling systems interact to modulate the excitability of neurons in a physiological milieu that includes a wide range of non-neural agents that also can affect neuronal excitability? For several years we have been examining these questions, focussing on interactions between substance P (SP) and angiotensin II (AngII) on prevertebral sympathetic neurons of guinea-pigs and on cell lines expressing NK1 receptors for SP or AT1A receptors for AngII. Intracellular electrophysiological recordings of guinea-pig coeliac ganglion neurons strongly suggest that receptors for SP and AngII converge on common intracellular signal transduction pathways to inhibit the same potassium channels to increase neuronal excitability. Based on combined electrophysiological and confocal microscopic analyses, most of SP released from collaterals of unmyelinated visceral nociceptive afferents probably acts non-synaptically. Mathematical modelling of SP diffusion using realistic morphological parameters derived from electron microscopy and direct measurements of SP diffusion coefficients with fluorescence correlation spectroscopy (FCS) or raster image correlation spectroscopy (RICS) show that physiological rates of afferent stimulation can generate concentrations of SP from non-synaptic release sites that are well within the range to affect the excitability of sympathetic neurons. Recently we have been using a confocal microscope with a high-speed resonant scanner and highly sensitive avalanche photodiodes to image the movement of EGFP-linked AngII receptors in CHO cells at rates of 20-25 frames/s. In addition to showing a considerable degree of constitutive internalisation of the receptors, these images have revealed the remarkably mobile nature of the cell membrane and the receptors it contains. Taken together, our data and models suggest that the environment within which peptides interact with their receptors is highly complex, such that they rarely occur under equilibrium conditions. In real life, it is most probable that sympathetic neurons are nearly always exposed to neuropeptides, peptide hormones and other agents at concentrations that increase their excitability significantly above a nominal resting levels. Somehow, the central nervous system must take this into account when regulating the degree of preganglionic drive to the peripheral neurons. Similarly, within the dorsal horn of the spinal cord, non-synaptic peptidergic transmission has the potential to greatly modify the processes underlying nociception.