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EDITORIAL

Localised intracellular signalling in neurons

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The neuron is a fundamental communication device and in order to function this way it needs high-fidelity input–output mechanisms. Modulation of neuronal activity is achieved by a multitude of intracellular signalling cascades. These include neurotransmitters and neuromodulators acting through G protein coupled receptors (GPCRs), growth factor receptors and various Ca^{2+} influx or release mechanisms. Surprisingly, however, the number of second messengers used in these signalling cascades is rather limited, which inevitably results in a situation where different signalling pathways utilise the same second messengers within the same neuron in order to achieve very different, sometimes even opposite effects. One obvious example of a second messenger involved in concurrent intracellular signalling pathways can be found with intracellular Ca^{2+} signalling. There is a plethora of pathways that can lead to rapid intracellular Ca^{2+} signals (e.g. opening of voltage gated Ca^{2+} channels during action potential firing, release of Ca^{2+} from intracellular stores in response to GPCR activation, Ca^{2+} influx through ionotropic receptors or ligand-gated or sensory ion channels, store-operated Ca^{2+} entry, etc.). Such Ca^{2+} signals regulate processes as diverse as synaptic transmission, neuronal firing patterns, gene expression and apoptotic cell death. It is therefore clear that robust mechanisms enabling precise coupling of particular type of Ca^{2+} signals to specific Ca^{2+}-dependent outputs must be in place. This consideration is likely to be applicable to other intracellular signalling mechanisms, such as cyclic nucleotide signalling, phosphorylation, lipid messenger signalling, etc. Growing evidence suggests that the precise spatial localisation of intracellular signalling cascades in many cases underlies the fidelity and specificity of signalling. Indeed, these cascades are often arranged in multi-protein signalling complexes at micro- or nanodomains of the plasmalemmal or intracellular membranes.

Current developments in our understanding of localised neuronal signalling cascades were discussed at the symposium entitled 'Localised intracellular signalling in neurons' held on 1 July 2014 during the Physiological Society's annual meeting, Physiology 2014, in London. The first speaker, John Garthwaite (University College London, UK) summarised his group's efforts to ascertain spatial and temporal profiles of neuronal nitric oxide (NO) signalling. NO acts as a neurotransmitter, diffusible second messenger and chemical modulator of proteins in the mammalian nervous system (Garthwaite, 2008) but many aspects of NO signalling remain unanswered; these include such issues as how 'local' NO signals are, and what the real physiological concentrations of NO in neurons are. Several elegant approaches aimed at quantitatively evaluating localised NO signalling in neurons were described; these include a quantitative model (Roy *et al.* 2008), real-time imaging of NO-mediated signal transduction in target cells (Batchelor *et al.* 2010) and an optical NO-detection system based on an NO-sensitive reporter cell line (Wood *et al.* 2011). These studies concluded that as a synaptic transmitter, NO is likely to act very locally, within the bounds of a single synapse, and exist only at very low (picomolar) concentrations. Yet, when a population of densely packed neurones are stimulated simultaneously, NO acts as a volume transmitter. Read more about localised NO signalling in the symposium review published in this issue of *The Journal of Physiology* (Garthwaite, 2016).

Nikita Gamper (University of Leeds, UK) reported on the localised Ca^{2+} signals that activate the Ca^{2+}-activated Cl^- channel ANO1 (TMEM16A) in nociceptive sensory neurons. ANO1 channel activation leads to depolarisation and excites nociceptive neurons as these accumulate high intracellular Cl^- concentrations (Liu *et al.* 2010). ANO1 channels have relatively low Ca^{2+} sensitivity (Yang *et al.* 2008; Xiao *et al.* 2011) and, thus, require close co-localisation with the intracellular Ca^{2+} source. Gamper's group found that ANO1 channels in nociceptive dorsal root ganglion (DRG) neurons reside within signalling complexes assembled at the junctions between the plasma membrane and the endoplasmic reticulum (ER). The complexes include phospholipase C (PLC)-coupled GPCRs (e.g. bradykinin B_2 receptors) and ANO1 channels at the plasma membrane and the inositol 1,4,5-trisphosphate (IP_3) receptors at the ER (Jin *et al.* 2013). The latter serve as a Ca^{2+} source for ANO1 activation in response to GPCR triggering by specific inflammatory mediators, as described in the symposium review from the Gamper's group (Jin *et al.* 2016).

Mark Shapiro (University of Texas Health Science Center at San Antonio, TX, USA) continued the theme of localised regulation of neuronal ion channels, focusing on the regulation of M-type (KCNQ, Kv7) K^+ channels by the AKAP79/150 signalling complexes. His group discovered several mechanisms by which such complexes can regulate M channel activity and expression. One 'rapid' signalling pathway involves recruitment of PKC to the channels, their subsequent phosphorylation, and a decrease in the channel affinity for the plasma membrane phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2) (Bal *et al.* 2010; Zhang *et al.* 2011), which is necessary for M channel activity (Suh & Hille, 2002; Zhang *et al.* 2003). The multiprotein complex mediating this signalling cascade includes AKAP79/150, M_1 muscarinic receptors and M channel proteins. There is also a 'slow' pathway whereby KCNQ channel expression can be regulated by neuronal activity. This pathway involves activation of calcineurin (CaN) and nuclear factor of activated T-cells (NFAT) transcription factor orchestrated by AKAP79/150. The pathway requires Ca^{2+} influx specifically through L-type Ca^{2+} channels and both local and global Ca^{2+} signals (Zhang & Shapiro, 2012). Super-resolution stochastic optical reconstruction microscopy (STORM) was employed to resolve individual molecular interactions within the AKAP79/150 signalling complex. This topic is further discussed in the symposium review published in this issue of *The Journal of Physiology* (Zhang & Shapiro, 2016).

Paola Pedarzani (University College London, UK) discussed signalling

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microdomains controlling the activity of a Ca^{2+}-dependent K^+ current known as sl(AHP) in hippocampal CA1 pyramidal neurons. sl(AHP) is important for the control of repetitive firing, setting of neuronal firing range and neuronal gain (Andrade *et al.* 2012). Ca^{2+} influx from voltage-gated Ca^{2+} channels and Ca^{2+}-induced Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores provides the activity-dependent drive for this current (Stocker *et al.* 2004). sl(AHP) is modulated by a variety of second messenger pathways. For example, monoamine transmitters suppress sl(AHP) through a signalling pathway that involves cAMP and protein kinase A (PKA) activation. Paola reported her group's recent studies on the role of Ca^{2+}-stimulated adenylyl cyclases (ACs), AC1 and AC8, in the modulation of sl(AHP) by neurotransmitters using knock-out mice lacking both ACs, concluding that sl(AHP) channels might reside in several distinct signalling microdomains in pyramidal neurons. Thus, AC1 and AC8 were not necessary for the suppression of sl(AHP) by some monoaminergic transmitters. Conversely, both ACs were necessary for the modulation of sl(AHP) channels by NMDA receptors in response to synaptic activity associated with long-term synaptic plasticity.

Dermot Cooper (University of Cambridge, UK) continued the theme of Ca^{2+} and cAMP microdomains, focusing on adenylyl cyclases. Ca^{2+} and cAMP are the principal intracellular second messengers that regulate a plethora of cellular processes, yet these two second messengers also modulate each other's activities in a number of cross-talking signalling pathways. For example, Ca^{2+} arising from store-operated Ca^{2+} entry (SOCE) can regulate AC activity in non-excitable cells but this coupling does not seem to exist in neurons and cardiomyocytes, where L-type Ca^{2+} channels provide Ca^{2+} for AC modulation (Willoughby & Cooper, 2007). Dermot described different molecular tools that were developed for the dissection of the microdomain localisation and coupling of ACs in living cells, such as Ca^{2+} (based on GCamps) and cAMP (based on EPAC) fluorescent probes tethered to ACs. Application of these sensors revealed that Ca^{2+}-sensitive ACs report quite distinct Ca^{2+} and cAMP signals to those reported by Ca^{2+}-insensitive ACs even in response to the same type of stimulus. Furthermore, the use of such sensors allowed dissection of the signalling complex that mediates selective activation of AC8 by SOCE (Willoughby *et al.* 2010; Willoughby *et al.* 2012).

In conclusion, this Physiological Society symposium discussed emerging hot topics in localised intracellular signalling in neurons with particular attention to localised GPCR signalling cascades, localised NO, Ca^{2+} and cyclic nucleotide signalling, microdomain regulation of ion channels, plasma membrane–ER junctional microdomains and spatially restricted regulation of gene expression. These localisation mechanisms are critical for ensuring fidelity of neuronal communication and signalling.

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Additional information

Competing interests

None declared.