Parvalbumin interneurons in the dorsal horn: it's not all about GABA

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Words (900 limit): 899

References: 5

Parvalbumin containing inhibitory interneurons (PV+ INs) are a key element controlling co-ordinated activity of neuronal ensembles in the mammalian central nervous system (CNS). In the spinal cord, inhibitory PV+ INs are believed to gate tactile information arriving from Aβ and Aδ inputs to deep layers of the mature dorsal horn (DH). Indeed, loss or activation of PV+ INs has been shown to induce or suppress allodynia in mice, respectively (Petitjean *et al.*, 2015). Despite much being known about synaptic connectivity of PV+ INs in the forebrain, particularly the role of mutual synaptic connections (Bartos *et al.*, 2001), relatively little is known of their inputs in the DH. However, inhibitory PV+ INs in LII/III of the mouse DH do share some common properties with their forebrain counterparts: they are fast spiking, have a local axonal arbour releasing GABA; they differ as their output synapses are predominantly axo-axonic on to myelinated afferent axon terminals (Hughes *et al.*, 2012).

In the present issue of *The Journal of Physiology*, the study of Gradwell *et al.* (2017) shows that in *substantia gelatinosa* of the DH, PV+ INs do not receive a strong GABAergic input, rather they are predominantly inhibited by ionotropic glycine receptor-mediated synaptic currents. Indeed reminiscent of GABA's action in the brain, glycine-mediated inhibition gives rise to both synaptic (phasic) and tonic inhibition in DH PV+ INs with little contribution from $GABA_A$-mediated tonic inhibition. Gradwell *et al*. demonstrate that glycine-mediated synaptic currents are produced by the activation of α/β-heteromeric glycine receptors, as revealed by their reduced sensitivity to picrotoxin and lindane. Furthermore, extrasynaptically-located and tonically-activated glycine receptors also possess a heteromeric subunit stoichiometry, as these display single-channel properties reminiscent of recombinantly expressed α/β-heteromeric glycine receptors. These data provide the first compelling evidence for heteromeric extrasynaptic glycine receptors producing tonic current, previously only observed for homomeric receptors. In further experiments, Gradwell and colleagues elegantly confirm that the tonic glycine currents are augmented by application of glycine transporter (GlyT) blockers. Interestingly, co-application of GlyT1 and GlyT2 blockers results in a non-linear summation of tonic current amplitude, suggesting that receptors mediating tonic inhibition may not be saturated by glycine under 'normal' conditions. This tonic glycine current exerts a persistent inhibition on the intrinsic excitability of PV+ INs, as the blocker strychnine leads to increased action potential discharge. Conversely, increasing extracellular glycine concentrations by application of GlyT blockers reduces PV+ IN action potential discharge, functionally silencing PV+ INs following extracellular glycine accumulation. Compellingly, the activation of glycine receptors by endogenous release of glycine is capable of producing a similar inhibition of PV+

IN firing, suggesting this mechanism is not confined purely to a pharmacologically modified environment and illustrates the functional importance of this inhibition in regulating the excitability of the DH network. A potential confound is that GlyT2 is present at PV+ IN axon terminals (Petitjean *et al.*, 2015) and uptake of glycine leads to a net inward current of 2 Na^+, which in the presence of glycine would lead to depolarisation of axon terminals, opposing the actions of glycine shown by Gradwell *et al.* (2017) in somatodendritic regions. This may lead to a more complex picture, perhaps explaining some of the paradoxical effects of glycine homeostasis on pain sensitivity.

This study opens the field up to further research and has far reaching consequences for our understanding of integration and processing in the spinal cord, particularly in the inappropriate processing of innocuous sensory stimuli. Oscillatory activity within the *substantia gelatinosa* is of low frequency (5-10 Hz) and is reliant on the actions of GABA and glycine acting at their receptors as well as electrical signalling via gap-junctions (Asghar *et al.*, 2005), possibly suggesting a role for PV+ INs. A simple interpretation of the reliance of PV+ INs on glycine for inhibitory tone, and not GABA, would be that mutual GABAergic synaptic connections may not exist between pairs of PV+ INs in contrast to their counterparts in the forebrain. What has not been addressed as yet is whether PV+ INs co-release GABA and glycine. Co-release has been shown to occur in the ventral horn of the spinal cord at synapses between inhibitory INs and motorneurons and PV+ INs do possess GlyT2 (Petitjean *et al.*, 2015) and therefore may possibly co-release GABA and glycine. Taken together these data suggest that PV+ INs in the DH could form mutual glycinergic connections, which may result in synchronous activity via glycine neurotransmission. It remains unclear whether PV+ INs of the DH are electrically coupled through gap-junctions, further facilitating co-ordinated PV+ IN activity. The ability of PV+ INs to control extrinsic inputs to the DH, perhaps leading to generation of the locomotor rhythm within the DH local network, suggests a plausible role for them in synchronisation of neuronal activity. In the forebrain, PV+ INs are associated most strongly with gamma band rhythms (20-100 Hz), which are far faster than the intrinsic oscillations in the DH (5-10 Hz, Asghar *et al.* (2005)), and one attractive possibility is that the frequency of oscillations relies on the level of excitatory drive on to PV+ INs, which are lower in the DH (Hughes *et al.*, 2012) than in the forebrain (Bartos *et al.*, 2001). This would plausibly explain differences in oscillation frequencies. Given the importance of local inhibition in sensory information relay, the dominance of glycine receptor-mediated inhibition in PV+ INs over GABAergic mechanism may have far reaching impact on the design of novel therapeutics for pain.

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