Differential inhibitory signalling in the superficial and deep dorsal horn of the mouse spinal cord

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Neurons in the superficial (SDH; laminae I-II) and deep (DDH; laminae IV-VI) dorsal horn of the spinal cord receive synaptic inputs from the periphery via small and medium diameter afferent fibres, respectively. Both regions have well-established, although largely separate, roles in the spinal processing of pain signals. Despite this, fast synaptic inhibition is provided by glycine- and $GABA_A$- receptors in both regions under normal conditions and both receptor types been implicated in the onset and maintenance of pathological pain states. Until recently, glycine receptors (GlyRs) throughout the adult nervous system were thought to be composed of α1/β-subunits, however an unusual form of the GlyR, containing α3-subunits, has recently been identified in the SDH, but not the DDH (Harvey *et al.*, 2004). Moreover, tonic inhibitory drive mediated by glycine and $GABA_A$ receptors differs in the superficial and deep layers of the dorsal horn (Cronin *et al.*, 2004). To further understand the contribution of these two inhibitory transmitter systems in spinal pain processing, we compared the electrophysiological properties of synaptically located GlyRs and $GABA_A$Rs in the SDH and DDH. Mice (C57Bl/6, both sexes, P17-37) were anaesthetised (Ketamine, 100 mg/kg, i.p.) and decapitated. Transverse slices (300 um thick) were prepared from the spinal cord (L3-L5 segments) and voltage-clamp recordings were made from SDH and DDH neurons (CsCl internal; holding potential 70 mV; 23ºC). Strychnine-sensitive (1 um) glycinergic mIPSCs were recorded in the presence of tetrodotoxin (1 μM), CNQX (10 μM) and bicuculline (10 μM). Bicuculline-sensitive (10 μM) $GABA_A$ergic mIPSCs were recorded in the presence of tetrodotoxin (1 μM), CNQX (10 μM) and strychnine (1 μM). Glycinergic mIPSCs were detected in 23/35 SDH neurons, but were observed in all DDH neuron recordings (20/20). In contrast, $GABA_A$ergic mIPSCs were present on all (n = 15) neurons tested in the SDH, but on only 15/18 neurons in the DDH. Several properties of the two receptors also differed in the SDH and DDH. For example, glycinergic mIPSC amplitude was smaller (38.0 ± 3.2 *vs.* 55.5 ± 5.6 pA; $p < 0.05$), mIPSC decay time was slower (10.3 ± 0.5 *vs.* 5.1 ± 0.4 ms; $p < 0.05$), and mIPSC frequency was lower (0.24 ± 0.04 *vs.* 0.90 ± 0.17 Hz; $p < 0.05$) in SDH *vs* DDH neurones. In contrast, $GABA_A$ergic mIPSCs had similar amplitudes (15.6 ± 1.3 *vs.* 16.2 ± 2.2 pA) and frequencies (0.16 ± 0.06 *vs.* 0.18 ± 0.06 Hz); however, decay times were also slower (23.0 ± 2.4 *vs.* 12.3 ± 0.8 ms) in SDH *vs* DDH neurones. Interestingly, the mean single channel current underlying these mIPSCs, estimated using peak-scaled non-stationary noise analysis, was identical in both regions for GlyR (3.9 ± 0.7 pA, n = 11 *vs.* 3.8 ± 0.7, n = 8) and $GABA_A$R (1.6 ± 0.1 pA, n = 10 *vs.* 1.6 ± 0.2 pA, n = 11). These electrophysiological data were compared with results from real-time PCR analysis of the expression of GlyR subunits (α1-4 and β) in the SDH and DDH. The α1 subunit gene was highly expressed in both SDH and DDH, although levels were increased three-fold in the SDH. The β subunit was also highly expressed throughout the dorsal horn, however levels were three-fold higher in the DDH. The expression of α3 subunit was lower, relative to α1 and β subunits, but at three fold higher levels in the SDH *vs.* DDH. Together, these data indicate that glycine and $GABA_A$ receptors with differing physiological properties contribute to fast synaptic inhibition in deep and superficial regions of the mouse dorsal horn. These features are likely to influence pain processing differently in the SDH and DDH and provide a basis for selectively modifying inhibition in the two regions.

Cronin JN, Bradbury EJ, & Lidierth M. (2004) *Pain*, **112:** 156-63.
Harvey RJ, Depner UB, Wassle H *et al.* (2004) *Science*, **304:** 884-7.