Molecular and electrophysiological characterization of neuropeptide Y Y1 receptor-expressing neurons in the substantia gelatinosa of the spinal cord

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Neuropeptide Y (NPY) receptors are expressed in substantia gelatinosa (lamina II) neurons of the spinal cord. NPY reduces behavioral signs of acute and chronic pain, in part through activation of the NPY Y1 receptor (Y1R). However, the cellular mechanism of Y1R-mediated analgesia remains unclear. One outstanding question is whether they are expressed in inhibitory and/or excitatory neurons, and where they fit within the dorsal horn microcircuity of pain transmission and pain control, especially in the setting of chronic pain arising from tissue or nerve injury. Behavioral pharmacology and targeted neurotoxin studies from our laboratory support the hypothesis that Y1R-expressing neurons are excitatory. Furthermore, we report that Y1Rs co-exist with multiple markers of excitatory neurons such as Tlx3, calbindin, calretinin, and somatostatin, but not a widely accepted marker of spinal inhibitory interneurons, PAX2. Using patch-clamp electrophysiology in current clamp mode we recorded from lamina II neurons in para-sagittal slices from the spinal cord lumbar L4/L5 segment of adult mice. In randomly recorded unlabeled neurons, we observed firing patterns in the following ratios: tonic (35 %), initial burst (28 %), delayed and gap (15 %) and single (22 %). In Y1R- positive cells visualized from slices prepared from Y1R-eGFP mice, the majority of cells exhibited initial burst firing (90%) upon current injection, with a small percentage (10%) exhibiting tonic firing. These results were unexpected, as it is widely assumed that tonic and perhaps initial burst firing patterns represent inhibitory, GABAergic neurons. To determine whether initial burst and tonic firing of Y1-expressing neurons is associated with the expression of glutamate or GABA, single-cell RT-PCR analyses are underway using vGlut2 and GAD67 primers. Also, dorsal root stimulation (DRS) evoked constant-latency, putative monosynaptic EPSCs at A-δ recruiting strengths in Y1R-GFP neurons (n = 2), suggesting that they receive primary afferent input from A-δ sensory neurons. To determine the effects of NPY on Y1R-GFP neurons, NPY was applied locally to recorded neurons, resulting in outward whole-cell currents (n = 4). In current- clamp mode, DRS evoked action potentials were abolished by application of NPY, and this was accompanied by a hyperpolarizing shift of the resting membrane potential by ~10 mV. Both effects recovered after wash-out. Taken together, our data suggest that endogenous spinal NPY produces analgesia by inhibiting the excitability of excitatory interneurons in the dorsal horn that express Y1R.