

Regional effects of opioid receptor agonists in the spinal dorsal horn

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The spinal dorsal horn is the initial site within the CNS that afferent sensory information is processed, and as such represents a primary site for modulation of this input. The spinal dorsal horn is highly organised. Nociceptive fibres predominantly input into superficial (laminae I-II) and deep (lamina V) dorsal horn. In contrast, Laminae III and IV receive input predominantly from A β fibres carrying non-nociceptive information. Opioid receptors are situated throughout the dorsal horn and act to inhibit nociceptive processing particularly in superficial laminae. However, the effect of opioid receptors on the modulation of sensory processing within different regions throughout the dorsal horn is unclear. Multi-electrode arrays composed of 64 electrodes were used for the first time, to simultaneously record from sites throughout the dorsal horn and to examine the impact of opioid receptor activation on synaptic activity across the regions of the dorsal horn.

Male Sprague-Dawley rats (200g) were anaesthetised with urethane (2 mg/kg i.p.) and the spinal cords were rapidly removed and placed into ice cold Artificial Cerebrospinal Fluid (ACSF). All procedures were ethically reviewed and were in compliance with UK legislation. Once removed, 350 μ m slices were cut from the L4-L6 region of the spinal cord and placed onto 8x8 multi-electrode arrays at room temperature. Electrical activity from different regions of the dorsal horn was classified into three groups: lamina I-II, laminae III-IV and lamina V according to the classification of Rexed. All compounds were bath applied and the effect on evoked field potentials was expressed as percentage of the control response before application of compound.

Stimulation of the white matter overlying the medial edge of lamina I evoked field potentials throughout the dorsal horn. Capsaicin (10 μ M, n = 6) inhibited the field potentials to (mean \pm SEM) 87 \pm 4.5 %, of control, indicating that a component of the field potential was mediated by activation of small diameter, capsaicin sensitive primary afferents. The potentials were almost completely abolished by DNQX (10 μ M) suggesting that they were largely mediated by release of glutamate acting at AMPA / kainate receptors.

The μ opioid agonist, DAMGO (1 μ M) inhibited field potentials in the superficial and deep laminae with little effect on middle laminae. In laminae I-II and lamina V the field potential was reduced to 81 \pm 2.6 % and 78 \pm 3.6% of control, respectively (n = 15 & 13). In lamina III the field potential was unaffected (96 \pm 4.0 % of control; n = 15). In contrast, the δ opioid agonist DPDPE (100 nM) produced a graduated inhibition of field potentials through superficial to deep dorsal horn (n = 12). Field potentials in laminae I-II were inhibited to 92.6 \pm 2.4 %, in laminae III-IV to 84.6 \pm 2.7 % and in lamina V to 81.6 \pm 4.1 % of control. An alternative δ agonist, Deltorphin II showed a similar pattern of inhibition, though field potentials were attenuated to a lesser degree. Following application of Deltorphin II (1 μ M; n = 4-6) field potentials were 97.2 \pm 2.8%, 97 \pm 3 % and 96 \pm 4.1% of control in laminae I-II, laminae III-IV and lamina V respectively. The κ opioid agonist, U50488 had no effect on the amplitude of the field potential. Following application of U50488 (1 μ M; n = 9-10), field potentials were 102 \pm 2.3 %, 101 \pm 4.7 % and 96 \pm 4.2 % of control in laminae I-II, II-IV and V respectively.

These data demonstrate for the first time, the use of multi-electrode arrays to simultaneously record synaptic activity throughout the dorsal horn. The data demonstrate regional variations in the modulatory effects of opioid receptors through the dorsal horn. Thus mu opioid receptor activation appears to modulate synaptic activity predominantly in areas associated with the processing of nociceptive information and has little impact in areas associated with non-nociceptive sensory input. Conversely delta opioid activation appears to attenuate synaptic input throughout the dorsal horn with no distinction between areas associated with nociceptive and non-nociceptive processing. These methods therefore allow for the first time, the effects of drugs to be studied on functionally distinct regions of the spinal cord.