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Research Report

Primary afferent neurons express functional delta opioid receptors in inflamed skin

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ABSTRACT

Peripherally-restricted opiate compounds attenuate hyperalgesia in experimental models of inflammatory pain, but have little discernable effect on nociceptive behavior in normal animals. This suggests that activation of opioid receptors on peripheral sensory axons contributes to decreased afferent activity after injury. Previously, we reported that direct application of morphine to cutaneous receptive fields decreased mechanical and heat-evoked responses in a population of C-fiber nociceptors in inflamed skin. Consistent with reported behavioral studies, direct application of morphine had no effect on fiber activity in control skin. The aim of the present study was to determine whether mechanical responsiveness of nociceptors innervating inflamed skin was attenuated by direct activation of delta opioid receptors (DORs) on peripheral terminals. An ex vivo preparation of rat plantar skin and tibial nerve was used to examine effects of a selective DOR agonist, deltorphin II, on responsiveness of single fibers innervating inflamed skin. Electrical recordings were made eighteen hours after injection of complete Freund's adjuvant into the hindpaw. Deltorphin II produced an inhibition of the mechanical responsiveness of single fibers innervating inflamed skin; an effect blocked by the DOR-selective antagonist, naltrindole. The population of units responsive to deltorphin II was identified as consisting of C fiber mechanical nociceptors.

1. Introduction

Within skin, an intricate network of blood vessels, epithelial cells, immune cells, and sensory axons communicates through electrical and chemical signals. Opioid peptides are synthesized and released from keratinocytes as well as migrating immune cells (Slominski et al., 2011; Stein and Zollner, 2009; Wintzen et al., 2001). Mu, delta and kappa opioid receptors have been localized to various cell types (immune, keratinocytes, axons) in the skin (Bigliardi et al.,1998; Stein et al., 1990b; Coggeshall, et al., 1997). Although the receptors are present, it is difficult to demonstrate their function in healthy tissues.

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Efficacy of opioid receptor agonists is enhanced under inflammatory conditions (Stein and Zollner, 2009; Wenk et al., 2006). Peripherally-restricted opioids attenuate behavioral hyperalgesia in models of inflammatory pain, with the little effect in normal animals (Stein and Zollner, 2009), suggesting that activation of opioid receptors on sensory axons decreases afferent excitability. We found that locally-applied morphine inhibits mechanical and thermal responsiveness of nociceptors innervating inflamed skin while having no effects on fibers in healthy skin (Wenk et al., 2006). It is unknown, to what degree specific mu, delta, or kappa opioid receptors on nociceptors contribute to peripheral opioid analgesia. The objective of the present study was to investigate whether mechanical responsiveness of nociceptors innervating inflamed skin was attenuated by direct activation of peripheral delta opioid receptors (DORs). We report that application of deltorphin II, a DOR-selective agonist, decreased evoked activity of nociceptors innervating inflamed skin. This effect was prevented by co-application of naltrindole, a DOR-selective antagonist. These results provide electrophysiological evidence for the existence of functional delta opioid receptors on the peripheral processes of nociceptive fibers innervating inflamed skin.

2. Results

2.1. Distribution of single units

We have shown previously that peripheral axons of nociceptors innervating healthy skin are not sensitive to morphine (Wenk et al., 2006) or to selective delta or mu receptor agonists (Schramm and Honda, 2010), so all recordings were made from axons innervating inflamed skin. Compound action potential recordings were made from the tibial nerve at the start of experiments. Isolated single units were classified into fiber type

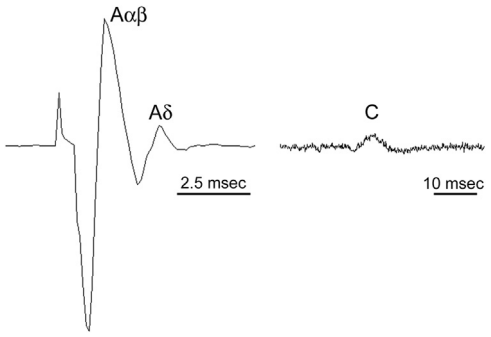

Fig. 1 – Basis for classification of single units by conduction velocity. Representative example of a compound action potential recording. Single units were classified into fiber types based on the conduction velocity range of compound action potential waveforms recorded for each preparation. Units with conduction velocities between the fastest part of the C wave and the slowest part of the Aδ wave were assigned to a fourth group, C/Aδ.The average range of conduction velocities for each waveform was as follows: Aα/β ($n=47$) 37.6±12.5 m/s to 9.5±3.0 m/s; Aδ ($n=44$) 11.7±3.9 m/s to 6.8±2.6 m/s; C ($n=18$) 0.72±0.15 m/s to 0.48±0.10 m/s.

(Aα/β, Aδ, or C) based on comparison of their conduction velocities to the range of conduction velocities of compound action potential waveforms for that experiment (Fig. 1). Ranges of conduction velocities (± standard deviation) for compound action potential waveforms were: Aα/β ($n=47$) 37.6±12.5 m/s to 9.5±3.0 m/s; Aδ ($n=44$) 11.7±3.9 m/s to 6.8±2.6 m/s; C ($n=18$) 0.72±0.15 m/s to 0.48±0.10 m/s. These averaged ranges of waveforms were used to classify single units in experiments where components of the compound action potential were not recorded. One standard deviation of the mean was added to the fastest part of each wave and one standard deviation was subtracted from the slowest part: units that conducted within 50.1 m/s and 6.5 m/s were classified as Aα/β fibers; units that conducted within 15.5 m/s and 4.2 m/s were classified as Aδ fibers; units that conducted within 0.87 m/s and 0.38 m/s were classified as C fibers. Units that conducted between the conduction velocity ranges of the Aδ and C waves were classified as C/Aδ fibers.

During isolation of single units, emphasis was focused on slowly conducting (<4.0 m/s) mechanical nociceptors. Forty units were included. Nineteen of these conducted within the C/Aδ fiber range with a mean conduction velocity (±standard deviation) 2.5 m/s±1.88, and twenty-one conducted within the C fiber range with a mean conduction velocity 0.58 m/s±0.14 (Table 1). Spontaneous activity was observed in 10 of 19 (52%) C/Aδ fibers and 15 of 21 (71%) C fibers (Table 1). The median mechanical threshold was 3.9 mN for C/Aδ fibers and 14.2 mN for C fibers. Eighteenof 19 C/Aδ fiber units (95%) and 20 of 21 C fiber units (95%) were functionally classified as nociceptors based on the pattern of their stimulus–response relationship (Table 1).

2.2. Vehicle

Responses of C and C/Aδ fibers to mechanical stimulation before and after application of vehicle were compared ($n=7$). The median baseline response to a 3.3 bar suprathreshold stimulus was 10.3 Hz (25th and 75th percentiles=3.0 and 11.6). The median response to a second mechanical stimulation after vehicle application was 14.8 Hz (25th and 75th percentiles=1.9 and 17.4). The pre- and post-vehicle responses were not significantly different from each other ($P>0.05$, Wilcoxon matched-pairs signed-ranks test).

2.3. Deltorphin II

As a combined population of C and C/Aδ fibers, responses of deltorphin II-treated units to mechanical stimuli were significantly inhibited as compared with vehicle treated fibers ($P<0.01$; one-way ANOVA). Mean percent baseline response (±SEM) at each concentration included: 1000 or 3000 nM ($n=6$, 71.8±25.2); 700 nM ($n=4$, 127.9±12.9); 300 nM ($n=5$, 49.9±7.8); 100 nM ($n=4$, 95.8±23.62); 10 nM ($n=3$, 39.5±21.6); 0.1 nM ($n=5$, 93.1±5.2); 0.001 nM ($n=6$, 100.3±14.3); vehicle ($n=7$, 123.2±11.6).

Previously we identified a population of morphine-sensitive primary afferent neurons as being C fiber nociceptors innervating inflamed skin (Wenk et al., 2006). In the present study, although the combined population of slowly conducting units was inhibited by deltorphin II, we also independently analyzed the effects of deltorphin II on C and C/Aδ fiber nociceptor