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Research Report

Morphine inhibits acid-sensing ion channel currents in rat dorsal root ganglion neurons

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ABSTRACT

Extracellular acidosis is a common feature in pain-generating pathological conditions. Acid-sensing ion channels (ASICs), pH sensors, are distributed in peripheral sensory neurons and participate in nociception. Morphine exerts potent analgesic effects through the activation of opioid receptors for various pain conditions. A cross-talk between ASICs and opioid receptors in peripheral sensory neurons has not been shown so far. Here, we have found that morphine inhibits the activity of native ASICs in rat dorsal root ganglion (DRG) neurons. Morphine dose-dependently inhibited proton-gated currents mediated by ASICs in the presence of the TRPV1 inhibitor capsazepine. Morphine shifted the proton concentration–response curve downwards, with a decrease of 51.4±3.8% in the maximum current response but with no significant change in the $pH_{0.5}$ value. Another μ-opioid receptor agonist DAMGO induced a similar decrease in ASIC currents compared with morphine. The morphine inhibition of ASIC currents was blocked by naloxone, a specific opioid receptor antagonist. Pretreatment of forskolin, an adenylyl cyclase activator, or the addition of cAMP reversed the inhibitory effect of morphine. Moreover, morphine altered acid-evoked excitability of rat DRG neurons and decreased the number of action potentials induced by acid stimuli. Finally, peripheral applied morphine relieved pain evoked by intraplantar of acetic acid in rats. Our results indicate that morphine can inhibit the activity of ASICs via μ-opioid receptor and cAMP dependent signal pathway. These observations demonstrate a cross-talk between ASICs and opioid receptors in peripheral sensory neurons, which was a novel analgesic mechanism of morphine.

Abbreviations: ASICs, acid-sensing ion channels; DRG, dorsal root ganglion; I_{pH}, proton-gated current; TRPV1, transient receptor potential vanilloid type 1; PKA, protein kinase A; AC, adenylyl cyclase; 8-Br-cAMP, 8-bromo-cAMP; DAMGO, [D-Ala2,N-Me-Phe4, Gly5-ol]-enkephalin; ANOVA, analysis of variance.

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1. Introduction

Tissue acidosis is a prominent feature of multiple pathological conditions such as inflammation, ischemic stroke, infections, and cancer. Protons are produced or released by the injured tissues, resulting in tissue acidosis (Deval et al., 2010). It is well known that the local extracellular pH levels drop to 5.4 in acute inflammation and to 4.7 in fracture-related hematomas (Staruschenko et al., 2007; Steen et al., 1992). Severe ischemia also induces the reduction of pH to 6.3 or even lower (Smith et al., 1986; Zha, 2013). It has been demonstrated that accumulations of protons depolarize the terminals of nociceptive primary sensory neurons to cause pain sensation, and that the depolarization is caused by a direct activation of proton gated ionic channels (Deval et al., 2003; Steen et al., 1995). Direct application of an acidic solution into the skin induces non-adapting pain (Jones et al., 2004; Steen et al., 1995). Although both acid-sensing ion channels (ASICs) and transient receptor potential vanilloid receptor type 1 (TRPV1) could be involved, studies have suggested that ASICs, rather than TRPV1, mediate pain sensation induced by acid injection (Deval et al., 2008; Wemmie et al., 2006). Acidosis-induced pain is suppressed by APETx2, a specific blocker of ASIC3, or knockdown of ASIC3 with siRNA (Deval et al., 2008). ASICs inhibitors have been shown to relieve pain in a variety of pain syndromes (Deval et al., 2010; Dube et al., 2009). Thus, ASICs emerge as a potential therapeutic target for pain therapy (Kweon and Suh, 2013; Wemmie et al., 2013).

ASICs are pH sensors for detecting a wide range of pH fluctuations during pathological conditions. To date, seven subunits of ASICs (1a, 1b1, 1b2, 2a, 2b, 3, and 4) encoded by four genes have been identified (Deval et al., 2008). All other ASICs, except ASIC4, are present in primary sensory neurons of the trigeminal, vagal, and dorsal root ganglia (Alvarez de la Rosa et al., 2002; Benson et al., 2002). In peripheral sensory neurons, ASICs have been found on cell bodies and sensory terminals, where they have been suggested to be important for nociception and mechanosensation (Price et al., 2000, 2001). Among the ASIC subunits, ASIC3, which is the most essential pH sensor for pain, is specifically localized in nociceptive fibers innervating the skeletal and cardiac muscles, joints, and bone (Ikeuchi et al., 2008; Noel et al., 2010). It has been shown that inflammation and tissue injury increase the expression levels of ASICs mRNA in dorsal root ganglion (DRG) neurons (Voilley et al., 2001). Moreover, the activity of ASICs is upregulated by pro-inflammatory mediators such as serotonin, bradykinin, arachidonic acid and nitric oxide (Cadiou et al., 2007; Qiu et al., 2012; Smith et al., 2007; Yan et al., 2013). ASICs are also the subject of various pharmacological actions. For example, ASICs are inhibited by cannabinoids and nonsteroid anti-inflammatory drugs (Liu et al., 2012; Voilley et al., 2001).

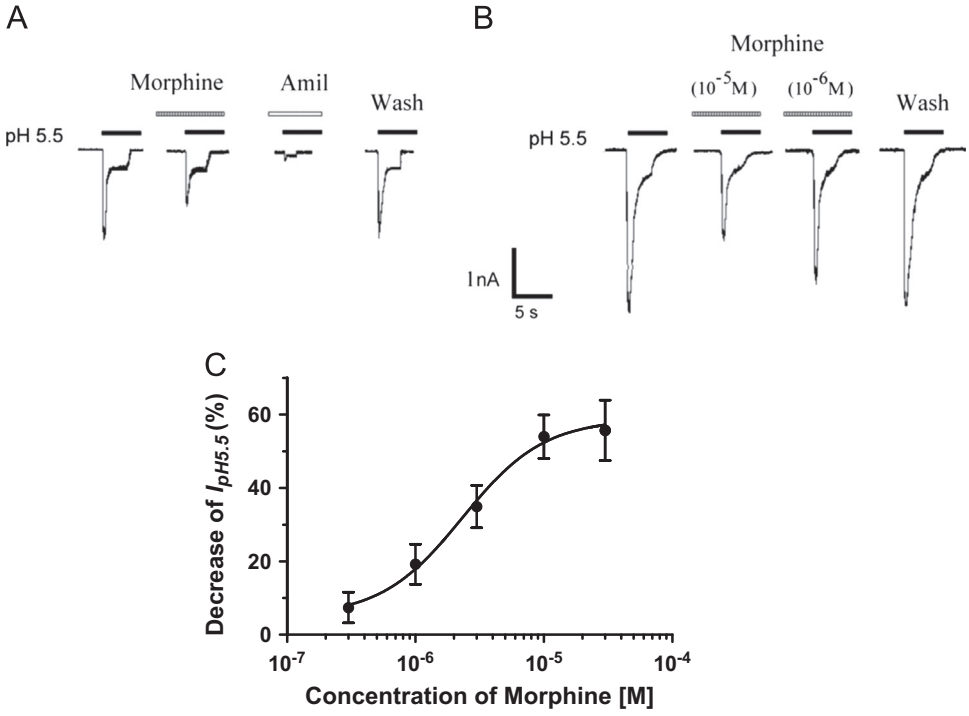

Fig. 1 – Concentration-dependent inhibition of the proton-gated current by morphine in rat DRG neurons. The proton-gated currents were recorded in DRG neurons. (A) Example traces show that the current was inhibited by pre-application of 10^{-5} M morphine for 60 s. Also, this proton-induced current could be completely blocked by 10^{-4} M amiloride (Amil), a broad-spectrum ASIC channel blocker. (B) Sequential current traces illustrate the inhibition of proton-induced currents by different concentrations of morphine on a DRG neuron with membrane potential clamped at −60 mV. (C) Morphine decreased proton-gated currents ($I_{pH\ 5.5}$) in a concentration-dependent manner (3×10^{-7}–3×10^{-5} M). Each point represents the mean ± SEM of 6–9 neurons. Proton-induced currents were evoked by extracellular application of a pH 5.5 solution for 5 s in the presence of the TRPV1 inhibitor capsazepine (10 μM).