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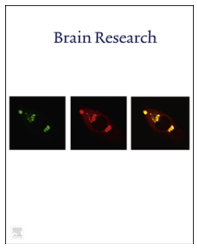

Research Report

Evidence for the participation of Ca^{2+}-activated chloride channels in formalin-induced acute and chronic nociception

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ABSTRACT

In this study we determined the role of Ca^{2+}-activated chloride channels (CaCC) in acute and chronic nociceptive responses elicited by 1% formalin. Formalin injection produced a typical pattern of flinching behavior for about 1 h. Moreover, it produced secondary allodynia and hyperalgesia in the ipsilateral and contralateral paws for at least 6 days. Local peripheral and intrathecal pre-treatment (−10 min) with the non-selective and selective CaCC blockers niflumic acid and $CaCC_{inh\text{-}A01}$, respectively, prevented formalin-induced flinching behavior mainly during phase 2 of the formalin test. Furthermore, niflumic acid and $CaCC_{inh\text{-}A01}$ also prevented in a dose-dependent manner the long-lasting evoked secondary mechanical allodynia and hyperalgesia in the ipsilateral and contralateral paws. Moreover, local peripheral and intrathecal post-treatment (on day 6) with both CaCC blockers decreased the established formalin-induced secondary mechanical allodynia and hyperalgesia behavior in both paws. CaCC anoctamin-1 and bestrophin-1 were detected in the dorsal root ganglia. Formalin injection increased anoctamin-1, but not bestrophin-1 protein levels at 6 days. Intrathecal injection of the CaCC inhibitor $CaCC_{inh\text{-}A01}$ prevented formalin-induced anoctamin-1 increase. Data suggest that peripheral and spinal CaCC, and particularly anoctamin-1, participates in the acute nociception induced by formalin as well as in the development and maintenance of secondary mechanical allodynia and hyperalgesia. Thus, CaCC activity contributes to neuronal excitability in the process of nociception induced by formalin.

Abbreviations: AUC, area under the curve; CaCC, Ca^{2+}-activated chloride channels; Cl^-, chloride; $[Cl^-]_i$, intracellular Cl^- concentration; CNS, central nervous system; DIDS, 4,4′-diisothiocyano-2,2′-disulfonic stilbene; DMSO, dimethyl sulfoxide; DRR, dorsal root reflexes; NKCC1, Na^+-K^+-$2Cl^-$ cotransporter 1; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid; PAD, primary afferent depolarization

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1. Introduction

Thermal or mechanical hyperalgesia and allodynia are common features of pain sensitization (Basbaum et al., 2009). Pain occurs in response to various inflammatory mediators such as prostaglandins, histamine, bradykinin, ATP, serotonin and cytokines, among others that are released from inflamed or injured tissues (Millan, 1999). A common feature of many of these mediators is that they increase intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) within the sensory terminals and would activate Cl^- currents producing a depolarizing inward current mediated by Cl^- efflux (Currie et al., 1995; Nicolson et al., 2002; Linhart et al., 2003).

Under physiological conditions, the reversal potential of Cl^- currents is around −40 mV ($[Cl^-]_i$=20–30 mM) among the most sensory neurons due to the expression and activity of Na^+–K^+–$2Cl^-$ cotransporter 1, NKCC1 (Sung et al., 2000). In these conditions, Cl^- efflux evokes an inhibitory mechanism known as primary afferent depolarization (PAD) through voltage-sensitive Na^+ channel inactivation that allows membrane voltage shunting and suppresses action potential propagation reducing pain sensation (Cervero and Laird, 1996; Cervero et al., 2003). Accordingly, Ca^{2+} influx and transmitter release decline (Rudomin and Schmidt, 1999). However, following tissue injury, Cl^- reversal potential shifts toward a more depolarized potential (up to −20 mV) increasing two or threefold the $[Cl^-]_i$ (Pieraut et al., 2007), which enhances the PAD to a level that may reach a threshold for generating action potentials called dorsal root reflexes (DRR), and the subsequent peripheral and central sensitization (Rees et al., 1995; Lin et al., 1999; Willis, 1999). Emerging evidence suggests that the activation of Ca^{2+}-activated chloride channels (CaCC) would be participating to generate this phenomenon.

CaCC are transmembrane proteins activated by intracellular Ca^{2+} concentration (Hartzell et al., 2005; Duran et al., 2010). CaCC are fundamental mediators in numerous physiological processes including transepithelial secretion, neuronal excitation and sensory transduction. The bestrophins were the first channels to settle within this family (Petrukhin et al., 1998; Sun et al., 2002; Tsunenari et al., 2003). So far, four genes encoding bestrophins in humans have been identified (bestrophin-1–4) (Tsunenari et al., 2003; Hartzell et al., 2008). The anoctamins (also called TMEM16) were discovered as part of the CaCC family in 2008 (Caputo et al., 2008; Yang et al., 2008). Ten genes encoding anoctamins have been proposed (Huang et al., 2012). CaCC were first recorded and characterized in sensory neurons from rat dorsal root ganglia (Mayer, 1985; Bader et al., 1987; Bernheim et al., 1989). Later, it was reported that axotomy induces up-regulation of CaCC in dorsal root and nodose ganglion neurons (Lancaster et al., 2002; André et al., 2003; Al-Jumaily et al., 2007; Boudes et al., 2009). Meanwhile knockdown of anoctamin-1 (ANO1) by using siRNA (Cho et al., 2012) or ablation of *Ano1* (Lee et al., 2014) promotes acute thermal and inflammatory pain. Additionally, a previous study reported that bradykinin produces its nociceptive effect through activation of CaCC while local injection of CaCC inhibitors attenuates bradykinin-induced nociception (Liu et al., 2010). Inflammatory mediators such as histamine activate inward Cl^- currents in dorsal root ganglion neurons (Cho et al., 2012). Since formalin is able to release bradykinin, histamine and serotonin (Parada et al., 2001), we hypothesized that formalin may activate CaCC to produce its nociceptive effect. Thus, in this study, we assessed the role of CaCC in acute nociception and long-lasting evoked secondary mechanical allodynia and hyperalgesia elicited by formalin. Furthermore, we determined the expression of the CaCC bestrophin-1 and anoctamin-1 in dorsal root ganglia of rats.

2. Results

2.1. *Effects of CaCC blockers in formalin-induced acute nociception*

Subcutaneous 1% formalin injection into the right hind paw produced a typical pattern of flinching behavior characterized by a biphasic time course. Phase 1 of the nociceptive response began immediately after formalin administration and then declined gradually in approximately 10 min. Phase 2 began about 15 min after formalin administration and lasted about 1 h. Administration of vehicle did not affect formalin-induced nociception (Fig. 1A,B).

The role of CaCC in the acute behavioral responses to subcutaneous injection of formalin was investigated by the injection of non-selective and selective CaCC blockers, niflumic acid and $CaCC_{inh\text{-}A01}$, respectively, with 1% formalin. Ipsilateral local peripheral (Fig. 1A) or intrathecal (Fig. 1B) administration of niflumic acid and $CaCC_{inh\text{-}A01}$ (1 μg/paw or 1 μg/rat), partially prevented formalin-induced acute nociception during phase 2 but not phase 1. Analysis of the area under the curve (AUC) showed that ipsilateral local peripheral or intrathecal injection of niflumic acid (Fig. 1C,D) and $CaCC_{inh\text{-}A01}$ (Fig. 1E,F) significantly ($P<0.05$) diminished AUC indicating an antinociceptive effect. In contrast, contralateral local peripheral administration of the drugs did not modify formalin-induced nociception (Fig. 1C,E).

2.2. *Effects of CaCC blockers in formalin-induced chronic nociception*

Besides producing acute nociception, 1% formalin injection elicited secondary mechanical allodynia and hyperalgesia in both paws. This was observed as a bilateral increase in paw withdrawal responses to the application of von Frey filaments (10 for allodynia and 250 mN for hyperalgesia) (Fig. 2). In order to determine if CaCC are involved in the development of these long-lasting evoked nociceptive behaviors, animals received a pre-treatment (−10 min) with the CaCC blockers before 1% formalin injection into the right (ipsilateral) paw. Animals were kept and evaluated six days later for secondary mechanical allodynia and hyperalgesia. Ipsilateral, but not contralateral, local peripheral pre-treatment with niflumic acid (Fig. 2A,B) (0.0001–1 μg/paw) or $CaCC_{inh\text{-}A01}$ (Fig. 2C,D) (0.001–1 μg/paw) fully prevented in a dose-dependent fashion formalin-induced long-lasting evoked secondary mechanical allodynia and hyperalgesia in both paws ($P<0.05$).