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Non-steroidal anti-inflammatory drugs attenuate agonist-evoked activation of transient receptor potential channels

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ABSTRACT

Transient receptor potential (TRP) cation channels are the largest group of sensory detector proteins expressed in the nerve terminals of many receptors including nociceptors, and are activated by temperature and chemicals that elicit hot or cold sensations. Antagonists of these channels are likely promising targets for new analgesic drugs at the peripheral and central levels. Because some non-steroidal anti-inflammatory drugs (NSAIDs) are structural analogs of prostaglandins and NSAIDs attenuate heat nociception and mechanical allodynia in models of inflammatory and neuropathic pain, we investigated whether three widely used NSAIDs (diclofenac, ketorolac, and xefocam) affect thermal and mechanical hyperalgesia following the activation of TRPA1 and TRPV1 channels. We measured nociceptive thermal paw withdrawal latencies and mechanical thresholds bilaterally at various time points following intraplantar injection of the TRPA1 agonists, cinnamaldehyde (CA) and allyl isothiocyanate (AITC) or the TRPV1 agonist capsaicin, or vehicle. When pretreated with vehicle, intraplantar injection of CA, AITC and capsaicin each resulted in significant decreases in thermal withdrawal latency and mechanical threshold in the ipsilateral hindpaw that did not return to baseline for more than 2 h. To test effects of NSAIDS either diclofenac, ketorolac or xefocam was pre-injected in the same hindpaw 35 min prior to CA, AITC or capsaicin. Pretreatment with each of the three NSAIDs produced strong antinociceptive and anti-hyperalgesic effects lasting approximately 60 min. Thus, we show for the first time that local administration of NSAIDs reduces thermal and mechanical hyperalgesia following TRPA1 or TRPV1 activation.

1. Introduction

Transient receptor potential (TRP) cation channels serve as cellular sensors for a wide spectrum of physical and chemical stimuli, such as temperature, cyclic nucleotides, phosphorylation potential, osmotic pressure, and some beneficial and harmful environmental inputs [1–11]. Recent studies have established the role of temperature-sensitive TRP (thermo-TRP) channels as molecular thermometers in the peripheral and central nervous system and have provided molecular insights into the mechanisms underlying the exquisite cold and heat sensitivity of these channels [1,2,7,12,13].

Chronic pain remains a medical and social problem worldwide. Although new agents for pain have been developed in the past few decades, for example newer non-steroidal anti-inflammatory drugs (NSAIDs) and cyclo-oxygenase-2 (COX-2) inhibitors, the current mainstay of therapy are agents such as opiates and NSAIDs [14–16]. NSAIDs are COX inhibitors. NSAID-mediated inhibition of COX results in inhibition of prostaglandin synthesis (PGs). PGs in turn cause sensitization and enhance pain signals in spinal neuronal circuits [17,18]. PGs are bioactive compounds of prostanoids, which include prostacyclin and thromboxane. Prostanoids originate from arachidonic acid, which is released intra-cellularly from plasma membrane phospholipids upon tissue damage and inflammation. Constitutively expressed COX-1 and inducible COX-2 convert arachidonic acid to the precursors PGG_2 and PGH_2, from which all prostanoids are generated by tissue-specific synthases [19]. The main mechanism of action of aspirin and NSAIDS is through the inhibition of COX activity, a discovery that was made in the early 1970s [20,21].

A number of arachidonic acid derivatives, including several electrophilic prostaglandins, have been shown to activate TRPA1 channels and covalent modification is thought to be the main mechanism underlying the direct activation of TRPA1 channels by these arachidonic acid derivatives, because the non-electrophilic precursors of these prostaglandins failed to show the same effect [22–25]. However, it is not known whether the overall structure of prostanoids also contribute to channel regulation. It was recently shown that pyrazolone derivatives selectively inhibited calcium currents in TRPA1-expressing cells and acute behavior responses in mice evoked by channel agonist, allyl

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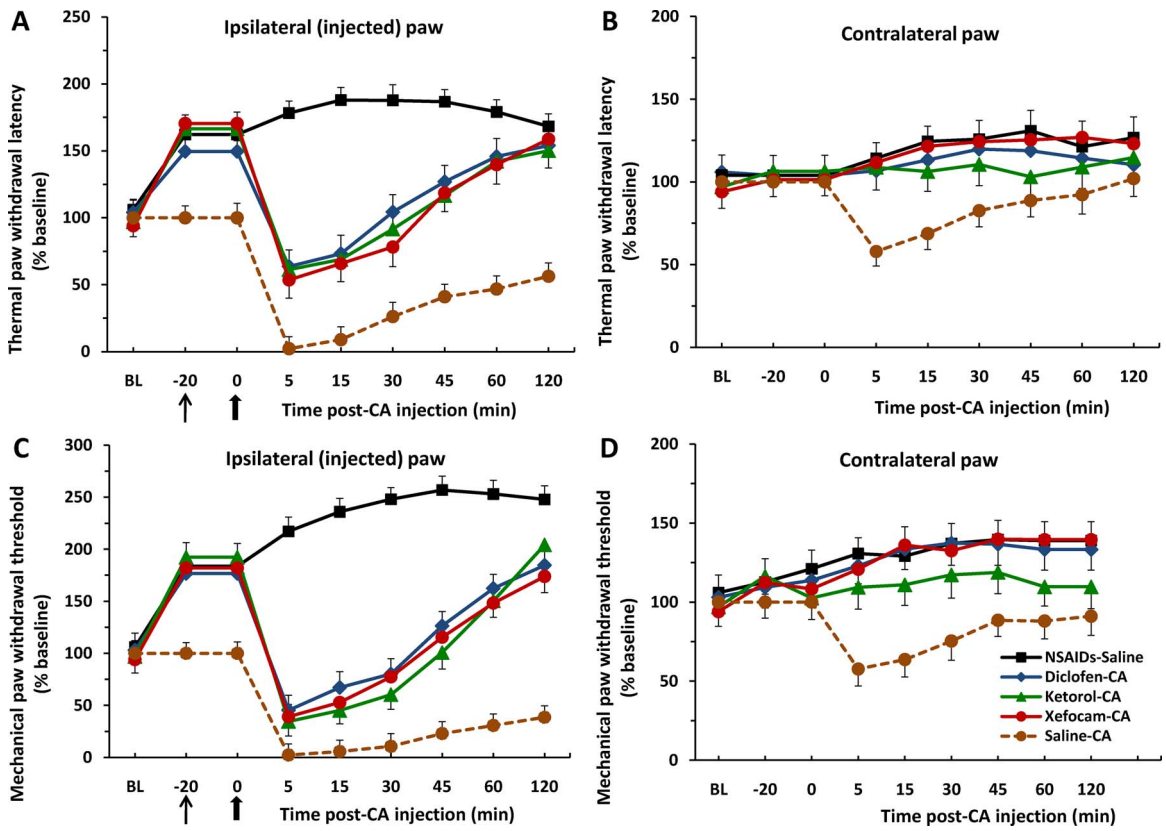

Fig. 1. (A) Dynamics of the thermal paw withdrawal latency after NSAIDs pretreatment following ipsilateral intraplantar injection of CA and vehicle. There are significant effects in CA groups *vs.* vehicle control and contralateral (non-injected) paw (B) for the first 30 min ($P < 0.001$). (C,D) The same as in A and B for von Frey mechanically evoked withdrawal threshold of the injected and non-injected paws. Note pretreatment with saline following intraplantar injection of CA resulted in thermal and mechanical hyperalgesia that persisted beyond 2 h (the brown dashed line). The thin black arrow indicates the time of injection of NSAIDs and the bold arrow indicates the time of injection of CA. BL – pre-injection baseline.

isothiocyanate (AITC) [26]. In other experiments hesperidin ("vitamin P") demonstrated antinociceptive activity and synergistic response when combined with ketorolac, possibly by involvement of the TRPV1 receptor ion channel, suggesting their clinical potential in pain therapy [27].

Here we report, using behavioral tests in rats that some commonly used NSAIDs, such as diclofenac, ketorolac, and xefocam probably inactivate or desensitize TRPA1 channel, to enable treatment with CA and AITC, and TRPV1 channel to enable treatment with capsaicin. A portion of this study was presented in a poster at the last World Congress of Pain [28].

2. Materials and methods

2.1. Animals

Behavioral studies were conducted using adult male Wistar rats (350–450 g), which were singly housed and given rodent chow and water *ad libitum*. The Beritashvili Experimental BMC Animal Care and Use Committee approved the study protocol (# 12/299-15). Every effort was made to minimize both the number of animals used and their suffering. Guidelines of the International Association for the Study of Pain regarding animal experimentation were followed throughout [29].

2.2. Application of chemicals

AITC (15%), CA (20%), and capsaicin (0.4%) (Sigma-Aldrich, St Louis, MO, USA) or vehicle control (mineral oil, or Tween 80, Fisher Scientific, USA, or saline, BioPharma, Ukraine) were injected intraplantar with a 30 gauge needle. For thermal and mechanical paw withdrawal tests, AITC, CA, capsaicin, or vehicle (2 µl) was applied to one hind paw. Thirty-five minutes prior to the start of the experiment, the same volume of NSAIDs, diclofenac (2.5%), ketorolac (3%), or xefocam (0.4%), or vehicle, was pre-injected in the same hindpaw and animals were examined by the thermal and mechanical paw tests. All these drugs are non-opioid medications, and are representative of three different groups of NSAIDs. Diclofenac is a derivative of phenyl-acetic acid, ketorolac belongs to the indole group and xefocam belongs to the group of oxicames. Different animal groups were used for the experiments and they were only tested with one concentration of chemicals (AITC, CA, capsaicin) or vehicle and were not repeatedly used. Six rats were used for each group. The NSAIDs doses were calculated by scaling human daily dosages to rat body weight [40].

2.3. Behavioral testing

Two behavioral models were used: thermal paw withdrawal (Hargreaves) test and mechanical paw withdrawal (von Frey) test (IITC, Woodland Hills, CA, USA). Prior to initiating the tests, baseline values were assessed for the experimental and control rats for thermal and mechanical withdrawal tests, which involved averaging five baseline measurements for the left and right hind paws with 5 min intervals.

2.4. Thermal paw withdrawal (Hargreaves) test

Rats were first habituated over three successive daily sessions to stand on a glass surface heated to 30 ± 1 °C within a ventilated Plexiglas enclosure. Before formal testing, baseline latencies for paw withdrawals evoked by radiant thermal stimulation were measured five times per paw, with at least 5 min intervals between tests of a given paw. A light beam (Plantar Test 390, IITC) was focused onto the plantar surface of the hindpaw through a glass plate from below, and the