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(+)-Borneol alleviates mechanical hyperalgesia in models of chronic inflammatory and neuropathic pain in mice

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ABSTRACT

Chronic pain is a major public health problem categorized as inflammatory or neuropathic, each involving impaired GABAergic control in the spinal cord of mammals. (+)-Borneol, a bicyclic monoterpene present in the essential oil of plants, is used for analgesia and anesthesia in traditional Chinese medicine. It has been reported that (+)-borneol directly potentiates GABA activity at recombinant human $GABA_A$ receptors. Although borneol has antinociceptive effect on acute pain models, little is known about its effect on chronic pain and its mechanism. Here we report that (+)-borneol has remarkable anti-hyperalgesic effects on neuropathic and inflammatory pain in animal models. Neuropathic hypersensitivity was induced by segmental spinal nerve ligation (SNL), and inflammatory hypersensitivity was induced by intraplantar (i.pl.) injection of complete Freund's adjuvant (CFA). Both oral administration (125, 250 or 500 mg/kg) and intrathecal injection (i.t.) (15, 30 and 60 μg) of (+)-borneol reduced mechanical hypersensitivity dose-dependently in SNL and CFA models. The anti-hyperalgesic effects of (+)-borneol were abolished by a selective $GABA_A$ receptor ($GABA_AR$) antagonist bicuculline (i.t., at 30 min after (+)-borneol injection). Furthermore, (+)-borneol (500 mg/kg, p.o. or 60 μg, i.t.) did not influence motor function. These findings suggest that (+)-borneol may ameliorate mechanical hyperalgesia by enhancing $GABA_AR$-mediated GABAergic transmission in the spinal cord, and could serve as a therapeutic for chronic pain.

1. Introduction

Chronic pain is a major public health problem, with epidemiological studies reporting about one fifth of the general population to be affected both in USA and Europe (Breivik et al., 2006). Different from acute pain conditions, chronic pain is categorized as inflammatory or neuropathic, each involving neuroplastic changes leading to hypersensitivity in both peripheral and central nociceptive system (Xu and Yaksh, 2011). Despite a lot of nonprescription analgesics being advertised and sold in drugstores, treatment of pain is still dominated by two classical medications: opioids and nonsteroidal anti-inflammatory drugs (Kissin, 2010). The treatment of chronic pain particularly associated with cancer or damage to the nervous system is at present inadequate (Okuse, 2007). Natural products with few side effects, such as monoterpenes, are emerging therapeutic resources for developing new drugs to treat chronic pain (Guimarães et al., 2013).

Central sensitization is important in the development and maintenance of chronic pain. After peripheral tissue or nerve injury, spinal cord dorsal horn inhibitory circuit is impaired, which serves as a key contributor to central sensitization (Scholz et al., 2005; Coull et al., 2003; Moore et al., 2002). Loss of inhibition contributes not only to the development of spontaneous pain but also to the mechanical hypersensitivity (allodynia) and hyperalgesia. γ-Aminobutyric acid (GABA) and glycine are the two fast inhibitory neurotransmitters in the mammalian spinal cord. Blocking spinal cord GABAergic neurotransmission by intrathecally injecting GABA receptor antagonist leads to hypersensitivity to innocuous stimuli (Suo et al., 2013; Cao et al., 2011). Drugs which activate $GABA_A$ receptors ($GABA_ARs$), such as $GABA_ARs$ agonists or positive allosteric modulators benzodiazepines, have been proposed as potent analgesics in various models of inflammatory and neuropathic pain (Knabl et al., 2008; Munro et al., 2009; Paul et al., 2014).

(+)-Borneol, a bicyclic monoterpene alcohol, is present in the essential oil of medicinal plants. Natural borneol is (+)-borneol and is widely used in food and also used for analgesia and anesthesia in traditional Chinese medicine (Hattori, 2000). Recent studies reported that (+)-borneol has a variety of pharmacological effects, including anti-inflammatory (Zhong et al., 2014) and neuroprotective effects (Q.S. Liu et al., 2011; R. Liu et al., 2011;). It has also been reported that (+)-borneol directly potentiates GABA activity at recombinant human $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors expressed in *Xenopus laevisoocytes* (Granger et al., 2005).

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Moreover, (+)-borneol is capable of preventing nociceptive behaviors induced by acetic-acid or formalin (Almeida et al., 2013). Despite being used in pharmaceutical preparations to treat acute pain, little is known about the effect of (+)-borneol on chronic pain. In the present study, we investigated whether (+)-borneol produces anti-hyperalgesic effect in chronic inflammatory and neuropathic pain in rodents, and possible mechanisms underlying the anti-hyperalgesic effect.

2. Materials and methods

2.1. Animals

All experimental protocols were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University. Male adult ICR mice (20–25 g) were purchased from the Experimental Animal Center of Nanjing Medical University throughout the study. The animals were housed at 22 ± 2 °C on a 12 h light/dark cycle with free access to water and food. The experiments were conducted after the mice were habituated to the testing environments for at least 5 days.

2.2. Segmental spinal nerve ligation-induced neuropathic pain

Neuropathic pain was induced by segmental spinal nerve ligation (SNL). The mice were anesthetized by intraperitoneal injection of 2% chloral hydrate (0.2 ml/10 g). After the loss of righting reflex, the mice were fixed in the prone position. The SNL model was prepared as described by the method of Chung et al. (2004): a median skin incision with about 3–5 cm length in L4-S2 level of the mouse back was made, the muscles next to the vertebrae till the sixth lumbar protruding were separated, the L5/L6 joint protruding on the right side was exposed and excised, and L6 processus transverse was partially splitted so that the L4–L6 spinal nerves on the right side were exposed. L5 nerve was gently isolated and tightly ligated with 5–0 silk thread. The wound was closed with 4–0 silk thread suture and covered with iodine solution. Mice with ligated nerves did not present signs of paw clonus or autotomy.

2.3. Complete Freund's adjuvant-induced inflammatory pain

Inflammatory pain was induced by injection of Complete Freund's adjuvant (CFA, 1 mg/ml of heat killed Mycobacterium tuberculosisin, 85% paraffin oil and 15% mannide monoleate) into planta, which is widely used in chronic inflammatory pain model. The skin of right planta was disinfected with 75% ethanol, and 10 μl CFA was injected subcutaneously into the planta of right hind limb. Animals with CFA injection exhibited foot swelling, thermal and mechanical hyperalgesia. The mechanical hypersensitivity was measured at 24 h after the injection. Ten min later mice were treated with (+)-borneol or vehicle.

2.4. Measurement of mechanical hypersensitivity

The threshold for 50% paw withdrawal (50% PWT) response to mechanical stimulus with von Frey filaments (Touch-Test™ Sensory Evaluator, North coast Medical, Inc.) was used to assess mechanical hypersensitivity. The 50% PWT refers to that the probability of paw withdrawal response to repeated mechanical stimulus is 50%. Mice were placed individually in a plastic cage (45 cm × 5 cm × 11 cm) with a wire mesh bottom which allowed full access to the paws. Behavioral accommodation was allowed for 20–30 min until cage exploration and major grooming activities ceased. Mechanical threshold was measured by applying a von Frey filament to the right hind paw until a positive sign of pain behavior was elicited. The paradigm for assessing the threshold was as follows. Von Frey filaments with logarithmically incremental stiffness (0.02–2 g) were applied serially to the paw by the up-down method (see Chaplan et al., 1994 for details and validation). The filaments were presented, in ascending order of strength, perpendicular to the plantar surface with sufficient force to cause slight bending against the paw and held for 4 s. A positive response was noted if the paw was sharply withdrawn. Flinching immediately upon removal of the hair was also considered a positive response. The 2-g filament was selected as the upper limit cut-off for testing. If there was no response at 2-g filament, animals were assigned this cut-off value. The pattern of positive and negative withdrawal responses was converted to 50% threshold according to the formula: 50% PWT $= 10^{\log\ (X)+\kappa\delta}$. X = value (in log unit) of the final von Frey hair used; κ is correction factors based on the pattern of responses from the calibration table; δ = mean difference in log units between stimuli (here, 0.224).

2.5. Drug administration

$GABA_A$ receptor antagonist bicuculline (Sigma) or (+)-borneol (Sigma) were intrathecally injected in 5-μl volume as previously reported (Hylden and Wilcox, 1980). Bicuculline was dissolved in saline. (+)-Borneol was dissolved in Tween 80 plus saline. The final concentration of Tween 80 was ≤ 10%. Drugs were given by intragastric administration (p.o.) or intrathecal injection (i.t.). All intrathecal injections were made at the L5–L6 intervertebral space using a 30-gauge 0.5-inch needle mounted on a 10-μl Hamilton syringe. A sudden advancement of the needle accompanied by a slight flick of the tail was used as the indicator for the proper insertion of the needle into the subarachnoid space. To make sure that the operators could perform this injection accurately, they were trained by injecting a similar volume of 1% methylene blue solution into subarachnoid before experiments. When the diffusion distance of dye to rostral and caudal cord was less than 1 cm from injection site, the injection was successful. Operators must achieve an accuracy of > 95% with dye injection before performing drug injection.

2.6. Statistical analysis

The statistical significance of differences between groups was determined by Kruska–Wallis ANOVA followed by Mann–Whitney U test for group pairwise comparison. P values < 0.05 were considered as indicative of significance. Data regarding the 50% mechanical thresholds determined by the up-down method were analyzed using the Kruska–Wallis test followed by Mann–Whitney U test for individual comparisons. Results were presented as mean ± S.E.M.

3. Results

3.1. (+)-Borneol reverses nerve ligation-induced neuropathic pain behaviors

We investigated the effect of (+)-borneol in a neuropathic pain model. Before SNL surgery, the baseline of 50% PWT was tested. As illustrated in Fig. 1(A) and (C), SNL surgery caused a marked development of mechanical hypersensitivity in the ipsilateral hind paw, 10 days after the surgical procedure. We found that (+)-borneol caused a significant increase in paw withdrawal threshold ipsilateral to the nerve injury when administered either p.o. (Fig. 1(**A**)) or i.t. (Fig. 1(**C**)). The paw withdrawal threshold was increased at 0.5 h both p.o. and i.t. administration. With p.o. administration, the effect of 500 mg/kg peaked approximately 2 h, and 125 or 250 mg/kg peaked