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# CONTRIBUTION AND INTERACTION OF KININ RECEPTORS AND DYNORPHIN A IN A MODEL OF TRIGEMINAL NEUROPATHIC PAIN IN MICE

**A. P. LUIZ,[a]* S. D. SCHROEDER,[a] G. A. RAE,[a] J. B. CALIXTO[a,b] AND J. G. CHICHORRO[c]**

[a] *Department of Pharmacology, Federal University of Santa Catarina, Florianopolis, SC, Brazil*

[b] *Center of Innovation and Pre-clinical Trials, Florianopolis, SC, Brazil*

[c] *Department of Pharmacology, Federal University of Parana, Curitiba, PR, Brazil*

**Abstract—Infraorbital nerve constriction (CION) causes hypersensitivity to facial mechanical, heat and cold stimulation in rats and mice and is a reliable model to study trigeminal neuropathic pain. In this model there is evidence that mechanisms operated by kinin $B_1$ and $B_2$ receptors contribute to heat hyperalgesia in both rats and mice. Herein we further explored this issue and assessed the role of kinin receptors in mechanical hyperalgesia after CION. Swiss and C57Bl/6 mice that underwent CION or sham surgery or dynorphin A (1–17) administration were repeatedly submitted to application of either heat stimuli to the snout or mechanical stimuli to the forehead. Treatment of the animals on the fifth day after CION surgery with DALBK ($B_1$ receptor antagonist) or HOE-140 ($B_2$ receptor antagonist), both at 0.01–1 μmol/kg (i.p.), effectively reduced CION-induced mechanical hyperalgesia. Knockout mice for kinin $B_1$, $B_2$ or $B_1$/$B_2$ receptors did not develop heat or mechanical hyperalgesia in response to CION. Subarachnoid dynorphin A (1–17) delivery (15 nmol/5 μL) also resulted in orofacial heat hyperalgesia, which was attenuated by post-treatment with DALBK (1 and 3 μmol/kg, i.p.), but was not affected by HOE-140. Additionally, treatment with an anti-dynorphin A antiserum (200 μg/5 μL, s.a.) reduced CION-induced heat hyperalgesia for up to 2 h. These results suggest that both kinin $B_1$ and $B_2$ receptors are relevant in orofacial sensory nociceptive changes induced by CION. Furthermore, they also indicate that dynorphin A could stimulate kinin receptors and this effect seems to contribute to the maintenance of trigeminal neuropathic pain.** 

**Keywords: kinin, dynorphin A, orofacial hyperalgesia, trigeminal neuropathic pain.**

## INTRODUCTION

The kallikrein-kinin system consists of precursor kininogens, proteolytic kallikrein enzymes, bradykinin (BK) and kallidin (produced through cleavage of kininogens by kallikreins), des-Arg$^9$-BK and des-Arg$^{10}$-kallidin (produced through cleavage of BK and kallidin, respectively, by exo-proteases) and two G-protein-coupled receptors (GPCRs), termed $B_1$ and $B_2$ receptors. These receptors mediate the pathophysiological actions of kinins, which include pain, inflammation, vasodilation, increased vascular permeability and natriuresis. The investigation of cardiovascular actions and the discovery of two receptor subtypes in the kinin field during the past 30 years have helped to identify the therapeutic potential of $B_1$ and $B_2$ receptor agonists and antagonists (Calixto et al., 2000; Marceau and Regoli, 2004). In this context, a pro-hyperalgesic role of $B_1$ and $B_2$ receptor agonists has been well demonstrated (Ferreira et al., 2002, 2004; Werner et al., 2007).

There is an extensive literature demonstrating the participation of kinin receptors in different models of neuropathic pain (Ferreira et al., 2005; Rajpal et al., 2007; Werner et al., 2007; Petcu et al., 2008; Quintao et al., 2008; Bujalska and Makulska-Nowak, 2009). However, little is known about their role in orofacial sensory alterations after trigeminal nerve injury. In this regard, our group has demonstrated that mechanisms operated by kinin $B_1$ and $B_2$ receptors contribute significantly to orofacial heat stimulation of rodents submitted to constriction of the infraorbital nerve (CION) (Luiz et al., 2010).

Interestingly, Lai and colleagues have shown that the endogenous opioid peptide dynorphin A contributes to the maintenance of hyperalgesia following injury to spinal nerves by acting as an agonist at kinin receptors (Lai et al., 2006, 2008). However, it is presently unknown if such an interaction of dynorphin A with kinin receptors also affects pain in models involving the trigeminal system.

Dynorphin A is thought to play a regulatory role in numerous functional pathways in the CNS. Indeed, dynorphin receptors are found in various brain structures, such as the hippocampus, amygdala, hypothalamus and striatum, and in the spinal cord, where they mediate actions of dynorphin related to learning and memory, emotional control, stress response and pain (Schwarzer, 2009). Additionally, upregulation of dynorphin A in the spinal cord has been consistently observed in experimental models of chronic pain (Malan et al., 2000; Wang et al., 2001; Lai et al., 2006). Considering the direct excitatory action of

*Corresponding author. Address: Molecular Nociception Group, Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT, UK. Tel: +44-20-7679-0793.
E-mail address: a.luiz@ucl.ac.uk (A. P. Luiz).

*Abbreviations:* BK, bradykinin; CION, constriction of the infraorbital nerve; DALBK, des-Arg$^9$-[Leu$^8$]-bradykinin; nor-BNI, nor-binaltorphimine dihydrochloride; PBS, phosphate-buffered saline.

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dynorphin A at kinin receptors, upregulation of this endogenous opioid has been shown to promote, rather than counteract, enhanced nociceptive input during inflammation (Luo et al., 2008).

In light of these considerations, the present study was carried out in mice to address the following points: a) the participation of $B_1$ and $B_2$ kinin receptors in orofacial mechanical and thermal hyperalgesia induced by chronic infraorbital nerve constriction; b) the ability of exogenous dynorphin A to induce orofacial sensory changes to heat and mechanical stimulation via kinin receptors and c) the effect of dynorphin A neutralization on CION-induced heat hyperalgesia.

## EXPERIMENTAL PROCEDURES

### Animals

Experiments were conducted on male Swiss mice weighing 30–40 g and female C57Bl/6 weighing 25–35 g. Animals were housed under conditions of optimum light and temperature (12 h light/dark cycle, lights on at 07:00 h, 22 ± 2 °C), with free access to laboratory chow and tap water. The experimental procedures were previously approved by UFSC's Committee on the ethical use of animals (Protocols 23080.009342/2008-11), where the study was carried out, and conducted in accordance with the ethical guidelines of the International Association for the Study of Pain – Zimmermann (1983) – and Brazilian regulations on animal welfare. All efforts were made to minimize the number of animals used and their suffering. Upon conclusion of the *in vivo* experiments, the animals were promptly sacrificed by $CO_2$ asphyxia in an acrylic chamber.

### CION

CION was produced according to the slight modification described in rats by Chichorro et al. (2006) using the method originally proposed by Vos et al. (1994). Briefly, mice were anesthetized with an intramuscular (i.m.) injection of a mixture of ketamine and xylazine (50 and 10 mg/kg, respectively) and an incision was made in the skin of the snout, under the right eye, about 3 mm caudal to the mystacial pads. The superior lip elevator and anterior superficial masseter muscles were bluntly dissected to expose the rostral end of the infraorbital nerve, as it emerged from the infraorbital fissure. Special care was taken not to damage the facial nerve. Two silk 4–0 ligatures were then tied loosely and 2 mm apart around the infraorbital nerve and the wound was closed with additional silk sutures. Sham-operated mice were operated identically, but no ligatures were applied to the nerve. After surgery, all mice were treated with oxytetracycline (500 mg/kg, i.m.) and maintained in a warm room until full recovery from anesthesia.

### Orofacial hyperalgesia induced by Dynorphin A (1–17)

To investigate the development of orofacial hyperalgesia induced by exogenous dynorphin, dynorphin A (1–17) was administered by subarachnoid (s.a.) medullary injection. To this effect, mice were briefly anesthetized with isoflurane (2% in 100% $O_2$), and a small area of the skin overlying the high cervical region was shaved with an electric razor. Dynorphin A (1–17) was administered at 15 nmol/site (Vanderah et al., 1996) between the occipital bone and C1 vertebra, as described previously by Fischer et al. (Fischer et al., 2005). This technique allowed direct drug delivery into the cerebrospinal fluid in the surroundings of the trigeminal subnucleus caudalis. Total injection volume in all experiments was 5 μL. Each animal regained consciousness approximately 30 s after discontinuing the anesthesia.

### Mechanical hyperalgesia assessment

For application of the mechanical stimulus, mice were acclimatized for 2 h individually in clear Plexiglas boxes (9 × 7 × 11 cm) on elevated wire-mesh platforms to allow access to the forehead and ventral surface of the right hindpaw. An initial behavioral assessment session was carried out in naïve mice to select them for further study according to their response to successive applications (10 consecutive times at ∼30 s-intervals) of von Frey filaments (Semmes–Weinstein Monofilaments, Stoelting, IL, USA) to the forehead (force 0.04 g), which receives trigeminal innervation and is easily accessible without need to restrict the animal, or the ventral surface of the right hindpaw (force 0.6 g). Attack/escape or head withdrawal reactions were considered positive responses to facial stimulation, whereas hindlimb withdrawal was the positive response end point to hindpaw stimulation. Only mice showing mean basal positive response frequencies of ∼25% to the 10 applications were used in the subsequent experiments.

To establish the time-course of CION-induced mechanical hyperalgesia, the mechanical stimulus was applied to the forehead and right hindpaw of sham-operated or CION-injured mice on the day preceding the surgery (to determine basal responsiveness) and then again on different postoperative days. Mechanical hyperalgesia was assessed daily after dynorphin A injection. In the experiments designed to investigate the effects of drug treatments on CION-induced mechanical hyperalgesia, the mechanical stimulus was applied solely to the forehead on the 5th and 36th days after surgery and the animals' responses were evaluated at 1-h intervals up to 4 h after the different treatments.

### Spontaneous nociception: face-grooming behavior

Mice were placed individually in the same clear Plexiglas boxes described above and a video camera was placed in front of the cage to enable continuous view of the animal's head. After a habituation period to the box for 15 min, behavior was recorded for 10 min and the time the animal engaged in facial grooming, i.e. when it contacted the facial region or grasped its ears with both forelimbs, was registered.

### Assessment of thermal hyperalgesia

Thermal heat hyperalgesia in the orofacial area was measured as previously described in rats (Almeida