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Basal μ-opioid receptor availability in the amygdala predicts the inhibition of pain-related brain activity during heterotopic noxious counter-stimulation

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ABSTRACT

The aim of this study was to investigate the association between the magnitude of anti-nociceptive effects induced by heterotopic noxious counter-stimulation (HNCS) and the basal μ-opioid receptor availability in the amygdala. In 8 healthy volunteers (4 females and 4 males), transcutaneous electrical stimulation was applied to the right sural nerve to produce the nociceptive flexion reflex (RIII-reflex), moderate pain, and scalp somatosensory evoked potentials (SEPs). Immersion of the left hand in cold water for 20 min was used as HNCS. In a separate session, basal μ-opioid receptor availability was measured using positron emission tomography with the radiotracer [^{11}C]carfentanil. HNCS produced a reduction of the P260 amplitude ($p < 0.05$), a late component of SEP that reflects activity in the anterior cingulate cortex. This reduction was associated with higher basal μ-opioid receptor availability in the amygdala on the right ($R^2 = 0.55$, $p = 0.03$) with a similar trend on the left ($R^2 = 0.24$, $p = 0.22$). Besides, HNCS did not induce significant changes in pain and RIII-reflex amplitude ($p > 0.05$). These results suggest that activation of μ-opioid receptors in the amygdala may contribute to the anti-nociceptive effects of HNCS. The lack of RIII-reflex modulation further suggests that μ-opioid receptor activation in the amygdala contributes to decrease pain-related brain activity through a cerebral mechanism independent of descending modulation.

1. Introduction

Heterotopic nociceptive counter-stimulation (HNCS) is an experimental procedure and a therapeutic intervention used to activate endogenous pain inhibition processes. HNCS involves the application of a sustained nociceptive stimulus, which inhibits nociceptive activity and pain induced by a competing nociceptive stimulus (LeBars et al., 1979; Willer et al., 1984; Chen et al., 1985; Kakigi, 1994; Piché et al., 2009; Moont et al., 2011). This analgesic response was initially described as diffuse noxious inhibitory controls in the rat (LeBars et al., 1979). It is also known as conditioned pain modulation in human (Yarnitsky et al., 2010). Although HNCS analgesia has been largely studied in both animals and humans, its mechanisms are still not completely understood.

In humans, HNCS can depress spinal nociceptive activity, indexed by the nociceptive flexion reflex (RIII-reflex) (Willer et al., 1984, 1989), although there are inter-individual differences in this anti-nociceptive response (Piché et al., 2009). The modulation of spinal nociceptive activity by HNCS depends, in part, on a spinobulbospinal loop that comprises the caudal region of the medulla (DeBroucker et al., 1990; Bouhassira et al., 1993) but may also involve the periaqueductal gray (PAG) (Piché et al., 2009). However, recent studies indicate that additional higher-order processes contribute to HNCS analgesia, including the activation of the

Abbreviations: ACC, anterior cingulate cortex; *DVR*, distribution volume ratio; EMG, electromyography; FWMH, full-width-half-maximum; HNCS, heterotopic noxious counter-stimulation; MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; NRS, numerical rating scale; OFC, orbitofrontal cortex; PAG, periaqueductal gray; PET, positron emission tomography; RIII-reflex, nociceptive flexion reflex; SEM, standard error of the mean; SEP, somatosensory evoked potentials.

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orbitofrontal cortex (OFC) (Piché et al., 2009; Moont et al., 2011) and the inhibition of nociceptive activity in the amygdala by the OFC (Piché et al., 2009).

The contribution of opioidergic neurotransmission to the anti-nociceptive effects of HNCS was shown in a pharmacological study using naloxone (Sprenger et al., 2011). Furthermore, a study on cerebral μ-opioid receptors in healthy humans indicates that μ-opioid receptors in the amygdala are activated during sustained pain (Zubieta et al., 2001). Besides, HNCS analgesia (Kosek and Hansson, 1997; Lautenbacher and Rollman, 1997; Julien et al., 2005) and the basal μ-opioid receptor availability in the amygdala (Harris et al., 2007) are decreased in patients with fibromyalgia. Altogether, these findings suggest that μ-opioid receptors in the amygdala may play a critical role in the regulation of pain and pain responses during HNCS. However, the relationship between basal μ-opioid receptor availability in the amygdala and inter-individual differences in anti-nociceptive responses induced by HNCS has not been examined in the same individuals.

The aim of this study was to examine the association between the anti-nociceptive effects of HNCS and μ-opioid receptor availability in the amygdala in healthy subjects. Transcutaneous electrical stimulation was applied in the territory of the sural nerve to produce the RIII-reflex, moderate pain, and pain-related somatosensory evoked potentials (SEP). We also examined cerebral μ-opioid receptor availability using positron emission tomography (PET) with the radiotracer [^{11}C]carfentanil in a separate session. We anticipated that HNCS would decrease pain ratings and SEP and we hypothesized that greater μ-opioid receptor availability in the amygdala would be associated with a greater reduction of pain and SEP by HNCS (main hypothesis). We also examined whether basal μ-opioid receptor availability in the amygdala was associated with the activation of descending inhibitory pathways during HNCS, as indexed by the reduction of RIII-reflex amplitude (second hypothesis).

2. Materials and methods

2.1. Ethics approval

All experimental procedures conformed to the standards set by the latest revision of the Declaration of Helsinki and were approved by the Human Research Ethics Committee of the Tokyo Metropolitan Institute of Gerontology. All participants gave written informed consent, acknowledging their right to withdraw from the experiment without prejudice and received a monetary compensation for their travel expenses, time and commitment to the study.

2.2. Study participants

Eight healthy right-handed volunteers were recruited in this study (4 females and 4 males; range 23–42 years old; mean ± SD: 28.5 ± 5.8 years old; BMI: 20.8 ± 0.6). All participants met the following inclusion criteria: (i) nonsmoker; (ii) no history of diseases that may affect the cardiovascular and/or nervous system such as heart diseases, diabetes mellitus, cancer; (iii) no history of acute or chronic pain; and (iv) no regular administration of therapeutic drugs or drug use on the day of the experiment. No participant had any organic abnormality in the brain, based on magnetic resonance imaging (MRI) scan examination. Participants were requested to fast for at least 2 h before the experiment, for both sessions (see Section 2.3). In addition, they were advised to avoid vigorous physical activity 3 h before the experiment and to abstain from alcohol during the 24 h preceding their participation. Females were tested in the follicular phase of their menstrual cycle to control for gender differences due to hormonal changes (range: 4–10 days after the onset of menses).

2.3. Study design

The study consisted in 2 sessions of approximately 120 min conducted on different days (separated by 1–3 days) to examine the correlation between the reduction of pain and SEP by HNCS and basal μ-opioid receptor availability in the amygdala. The modulation of pain, SEP and RIII-reflex by HNCS was assessed in the first session. In the second session, brain images were obtained using PET with the radiotracer [^{11}C]carfentanil to measure the basal μ-opioid receptor availability. MRI scans were also obtained for all participants to exclude brain abnormalities and for the analyses of PET images.

2.4. Painful electrical stimulation (test stimulus)

Transcutaneous electrical stimulation (trains of 10 × 1-ms pulses at 333 Hz) was delivered by a constant-current stimulator (Model DS7, Digitimer, Welwyn Garden, UK), triggered by a computer-controlled sequencer (Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK). Degreased skin over the retromalleolar path of the right sural nerve was stimulated by a pair of surface electrodes (model EL-258, Biopac Systems, Goleta, CA, USA) with an inter-electrode distance of 2 cm. Stimulus intensity was adjusted individually, according to the RIII-reflex threshold (see below).

2.5. Heterotopic noxious counter-stimulation (conditioning stimulus)

HNCS was produced by immersion of the left hand in circulating cold water during 20 min (CoolMan PAL C-307, Sibata Scientific Technology, Soka, Saitama, Japan). Water temperature was adjusted individually approximately 30 min before the experiment (range: 8–17 °C) to produce moderate pain (40–60 on a numerical rating scale (NRS)). HNCS pain and anxiety was rated every min and water temperature was adjusted manually as needed to maintain moderate pain during the 20-min period.

2.6. Pain and anxiety ratings

Pain and anxiety evoked by electrical stimulation of the sural nerve were rated verbally for every stimulus using two NRS ranging from 0 “no pain” or “no anxiety” to 100 “worst pain imaginable” or “worst anxiety imaginable” where 1 was defined as the pain/anxiety threshold. For both measures, a mean value was computed for each condition to compare baseline, HNCS and recovery periods. During HNCS, the same NRS were also used to rate HNCS pain and anxiety every min.

2.7. RIII-reflex measure and analysis

Electromyography (EMG) of the short head of the biceps femoris was recorded with a pair of EL-508 surface electrodes connected to an EMG100C amplifier (Biopac Systems, Goleta, CA, USA). EMG was amplified 2000 times, band pass filtered (10–500 Hz), sampled at 1000 Hz (Power 1401 acquisition system, Cambridge Electronic Design, Cambridge, UK) and stored on a personal computer for off-line analyses (Acqknowledge 4.1.1, Biopac Systems, Goleta, CA, USA). The RIII-reflex threshold was determined using the staircase method as described in previous studies (Willer, 1977; Piché et al., 2011; Ladouceur et al., 2012). The intensity of stimulation was then adjusted individually at 120% of the RIII reflex threshold and remained constant for the remainder of the experiment.