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SYMPATHETIC REGULATION AND ANTERIOR CINGULATE CORTEX VOLUME ARE ALTERED IN A RAT MODEL OF CHRONIC BACK PAIN

SARA TOUJ,[a,b] SÉBASTIEN HOULE,[a] DJAMEL RAMLA,[a] RENAUD JEFFREY-GAUTHIER,[a,b] HARUMI HOTTA,[a,d] GILLES BRONCHTI,[b,c] MARIA-GRAZIA MARTINOLI[e] AND MATHIEU PICHÉ[a,b]*

[a] *Department of Chiropractic, Université du Québec à Trois-Rivières, 3351 Boul. Des Forges, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada*

[b] *CogNAC Research Group, Université du Québec à Trois-Rivières, 3351 Boul. Des Forges, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada*

[c] *Department of Anatomy, Université du Québec à Trois-Rivières, 3351 Boul. Des Forges, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada*

[d] *Department of Autonomic Neuroscience, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan*

[e] *Department of Medical Biology, Université du Québec à Trois-Rivières, 3351 Boul. Des Forges, C.P. 500, Trois-Rivières, QC G9A 5H7, Canada*

Abstract—Chronic pain is associated with autonomic disturbance. However, specific effects of chronic back pain on sympathetic regulation remain unknown. Chronic pain is also associated with structural changes in the anterior cingulate cortex (ACC), which may be linked to sympathetic dysregulation. The aim of this study was to determine whether sympathetic regulation and ACC surface and volume are affected in a rat model of chronic back pain, in which complete Freund Adjuvant (CFA) is injected in back muscles. Sympathetic regulation was assessed with renal blood flow (RBF) changes induced by electrical stimulation of a hind paw, while ACC structure was examined by measuring cortical surface and volume. RBF changes and ACC volume were compared between control rats and rats injected with CFA in back muscles segmental (T10) to renal sympathetic innervation or not (T2). In rats with CFA, chronic inflammation was observed in the affected muscles in addition to increased nuclear factor-kappa B (NF-kB) protein expression in corresponding spinal cord segments ($p = 0.01$) as well as decreased ACC volume ($p < 0.05$). In addition, intensity-dependent decreases in RBF during hind paw stimulation were attenuated by chronic pain at T2 (p's < 0.05) and T10 (p's < 0.05), but less so at T10 compared with T2 (p's < 0.05). These results indicate that chronic back pain alters sympathetic functions through non-segmental mechanisms, possibly by altering descending regulatory pathways from ACC. Yet, segmental somato-sympathetic reflexes may compete with non-segmental processes depending on the back region affected by pain and according to the segmental organization of the sympathetic nervous system.

Keywords: nociception, somato-autonomic reflexes, sympathetic, chronic pain, anterior cingulate cortex, neuroinflammation.

INTRODUCTION

Chronic pain is highly prevalent in the adult population, affecting approximately 20% of individuals (Schopflocher et al., 2011). In addition to pain and disability, chronic pain syndromes are associated with autonomic disturbance (Chelimsky et al., 2001; Staud, 2008; Giannoccaro et al., 2014; Chelimsky et al., 2016). Consistent with these observations, animal and human studies on somato-autonomic reflexes show that nociceptive stimulation of the skin, muscles and joints can influence autonomic functions through somato-autonomic reflexes (Sato et al., 1997; Desmarais et al., 2011). However, the effects of chronic nociceptive inputs relevant for the physiopathology of chronic pain have been largely disregarded.

Acute nociceptive stimulation of the limbs generally produces non-segmental sympathetic reflexes integrated in brain structures, while nociceptive stimulation of the trunk may evoke segmentally organized sympathetic reflexes integrated in the spinal cord, in addition to non-segmental reflexes (Sato and Schmidt, 1971, 1973; Sato and Swenson, 1984; Kimura et al., 1995, 1996; Budgell and Suzuki, 2000; Piché et al., 2014). To our knowledge, only one study investigated somato-autonomic interactions in chronic conditions. In this study, nociceptive heat stimulation of a paw decreased skin temperature of the three non-stimulated paws. However, this response (vasoconstriction) was attenuated in rats with long-lasting hind paw hyperalgesia, reflecting sympathetic dysregulation (Vierck et al., 2008). To our knowledge,

*Correspondence to: M. Piché, Department of Chiropractic, CogNAC Research Group, Université du Québec à Trois-Rivières, 3351 boul. des Forges, C.P. 500, Trois-Rivières, Québec G9A 5H7, Canada. Fax: +1-819-376-5204.

E-mail address: mathieu.piche@uqtr.ca (M. Piché).

Abbreviations: ACC, anterior cingulate cortex; ANOVA, analysis of variance; CFA, complete Freund adjuvant; CTL, control; DLPFC, dorsolateral prefrontal cortex; IASP, International Association for the Study of Pain; MAP, mean arterial pressure; MCC, midcingulate cortex; NF-kB, nuclear factor-kappa B; PAG, periacqueductal gray matter; RBF, renal blood flow; RVM, rostral ventromedial medulla; SEM, standard error of the mean; T2, T6, T10, T13, thoracic segment 2, 6, 10, 13.

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however, the effects of long-lasting nociceptive activity on sympathetic regulation in animal models of back pain have not been investigated. Considering the segmental organization of somato-sympathetic reflexes, chronic back pain may produce specific sympathetic dysregulation or an interaction of segmental and non-segmental sympathetic processes, which may be of clinical relevance. Therefore, this interaction between chronic back pain and sympathetic regulation remains to be investigated.

In addition to its effects on sympathetic functions, chronic pain can produce maladaptive plasticity in various brain regions (Johansen et al., 2001; May, 2008; Zhuo, 2008, 2014; Blom et al., 2014; Lu et al., 2014; Newman et al., 2014). For example, changes in brain structure and function in prefrontal, insular and cingulate areas have been observed in several studies and they may be associated with physiological or functional impairment (Apkarian et al., 2004a,b; Schmidt-Wilcke et al., 2006; Siddall et al., 2006; Kuchinad et al., 2007; Lloyd et al., 2008; Metz et al., 2009; Wand et al., 2011; Saab, 2012; Piché et al., 2013; Blom et al., 2014). Among these regions, the anterior part of the cingulate cortex (ACC) is of particular interest in the study of autonomic dysregulation produced by chronic pain because it is involved in both pain and autonomic regulation (Mohr et al., 2005; Vogt, 2005; Qu et al., 2011). Indeed, ACC sends prominent projections to the somatosensory cortex, the amygdala, as well as to the periaqueductal gray matter (PAG) and spinal cord, structures that are involved in nociceptive and autonomic integration (Zhuo, 2014).

In the rat, injection of complete Freund's adjuvant (CFA) in musculoskeletal tissues is a common model of chronic pain and may be performed in the hind paw (Walker et al., 2003), tail (Vanegas and Schaible, 2004), joints (Masocha, 2013; Kaneguchi et al., 2016), or muscles (Ambalavanar et al., 2006; Chacur et al., 2009; Asgar et al., 2015). When injected in muscles or fascia, CFA induces chronic inflammation in the affected tissues, sensitization of dorsal horn neurons as well as neuroinflammatory changes in the corresponding spinal cord segments, including microglial activation (Chacur et al., 2009; Hoheisel and Mense, 2015). Interleukine-6 (IL-6), nuclear factor-kappa B (NF-kB) and Cyclooxygenases-2 (COX-2) are three critical markers of the complex process of neuroinflammation. IL-6 is a potent neuroinflammatory signal known to stimulate microglia and astrocytes to release a cascade of proinflammatory cytokines and acute phase proteins (Luo and Zheng, 2016). NF-kB is a critical immediate early response gene involved in modulating cellular responses and apoptosis following diverse injuries (Snow and Albensi, 2016). COX-2, together with COX-1, is a key enzyme in the conversion of arachidonic acid into bioactive prostanoids, playing a central role in the inflammatory cascade (Patrignani and Patrono, 2015). In addition, COX-1 and COX-2 are actively involved in neuronal dysfunction induced by pro-inflammatory stimuli (Yagami et al., 2016). Therefore, IL-6, NF-kB and COX-2 may contribute to sympathetic dysregulation and morphological changes in ACC that are associated with chronic back pain but this is still unknown. Considering the clinical implications of sympathetic dysregulation and ACC plasticity, this topic deserves further investigation.

The aim of the present study was to determine whether sympathetic regulation and ACC structure are altered in rats with chronic back pain. Sympathetic regulation was assessed with renal blood flow (RBF) changes induced by electrical stimulation of a hind paw, while ACC structure was examined by measuring the cortical surface and the volume. RBF changes and ACC structure were compared between control rats and rats with chronic back pain in regions segmental (T10) or not (T2) to renal sympathetic innervation. In addition, protein expression of three known markers of neuroinflammation, namely NF-kB, COX-2 and IL-6, was measured by Western blot analysis of spinal cord tissues.

We hypothesized that somato-renal reflexes would be attenuated in rats with chronic back pain compared with controls. However, we expected differential effects for rats with chronic pain affecting the lower (T10) or upper (T2) thoracic spine, in accordance with the segmental organization of renal sympathetic innervation. As an indication of neuroinflammation associated with these pathological changes, we also anticipated increased protein expression of IL-6, NF-kB and COX-2 in corresponding spinal cord segments. Finally, we hypothesized that ACC volume would be decreased in rats with chronic pain.

EXPERIMENTAL PROCEDURES

Ethical approval

Experiments were performed on 42 male Wistar rats (body weight 300–450 g, Charles River Laboratories International, Willmington, MA, USA). Animals were kept in local facilities with a light/dark cycle of 14 h/10 h. All experimental procedures were approved by the animal care committee of "Université du Québec à Trois-Rivières", in accordance with the guidelines of the Canadian Council on Animal Care and the guidelines of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP).

Chronic pain model and animal groups

Chronic back pain was induced by an intramusclar injection of complete Freund Adjuvant (CFA) (Difco Lab., Detroit, MI, USA), as described previously (Chacur et al., 2009). Under ultra-short isoflurane anesthesia, a volume of 150 μL was injected through the skin in thoracic paraspinal muscles, using a 27-gauge needle.

To examine the effect of chronic back pain on sympathetic regulation and brain morphology, experiments were conducted on 3 groups of rats. Rats from the control group (n = 16) received a 150 μL isotonic saline injection in T10 paraspinal muscles. Rats from the two other groups received a 150 μL injection of CFA (Difco Lab., Detroit, MI, USA) in T10 (CFA-T10, n = 16) or T2 (CFA-T2, n = 10) paraspinal muscles. Twelve days post-injection, ten animals from each of the three groups were used for the physiological experiment while six animals from the CFA-T10 group and the