Neuroscience 344 (2017) 157–166

THE NITRIC OXIDE DONOR, ISOSORBIDE DINITRATE, INDUCES A CEPHALIC CUTANEOUS HYPERSENSITIVITY, ASSOCIATED WITH SENSITIZATION OF THE MEDULLARY DORSAL HORN

JOSÉ MARÍA FLORES RAMOS,[a] LAURENT DEVOIZE,[a,b] AMÉLIE DESCHEEMAEKER,[a] JEAN-LOUIS MOLAT,[a] PHILIPPE LUCCARINI[a] AND RADHOUANE DALLEL[a,b]*

[a] *Université Clermont Auvergne, Inserm Neuro-Dol U1107, F-63000 Clermont-Ferrand, France*

[b] *CHU Clermont-Ferrand, Service d'Odontologie, F-63100 Clermont-Ferrand, France*

Abstract—Nitric oxide donors are known to produce headache in healthy as well as migraine subjects, and to induce extracephalic cutaneous hypersensitivity in rodents. However, little is known on the effect of nitric oxide donors on cephalic cutaneous sensitivity. Combining behavioral, immunohistochemical, and *in vivo* electrophysiological approaches, this study investigated the effect of systemic administration of the nitric oxide donor, isosorbide dinitrate (ISDN), on cephalic and extracephalic cutaneous sensitivity and on neuronal activation within the medullary dorsal horn (MDH) in the rat. Systemic administration of ISDN increased selectively the first phase and interphase of the facial formalin test, but had no effect on the hindpaw formalin one. Monitoring neuronal activity within the MDH with phospho-ERK1/2 immunoreactivity revealed that ISDN alone did not activate MDH neurons, but significantly increased the number of formalin-evoked phospho-ERK1/2-immunoreactive cells in the ipsilateral, but not contralateral, MDH. Using *in vivo* electrophysiological unit recordings, we show that ISDN administration never affected the spontaneous activity of trigeminal wide dynamic range neurons, but, facilitated C-fiber-evoked responses in half the neurons tested. This research demonstrates that a nitric oxide donor, isosorbide dinitrate, induces selectively cephalic hyperalgesia that arises as a consequence of central sensitization in pain pathways that subserve meningeal nociception. This model better mimics the clinical condition and offers another possibility of studying the role of nitric oxide donor in the physiopathology of headache.

Key words: headache, pain, migraine, hyperalgesia, central sensitization, trigeminal.

INTRODUCTION

Migraine is a common disorder affecting about 15% of the population (Stovner and Andree, 2010). Cutaneous hypersensitivity is a key feature of migraine (Bigal et al., 2008). It is a consequence of central sensitization in pathways that subserve meningeal pain (Bernstein and Burstein, 2012). Cutaneous hypersensitivity may occur within only the referred area of headache (that is the periorbital region) or spread throughout the face and scalp (Ashkenazi et al., 2007; Burstein et al., 2000; Cooke et al., 2007; Guy et al., 2010; Kitaj and Klink, 2005; Yoon et al., 2010) as well as the body and limbs (Burstein et al., 2000; Guy et al., 2010). About two-thirds of migraine patients exhibit cutaneous hypersensitivity over the course of their migraine attacks (Bigal et al., 2008). The prevalence and severity of cutaneous hypersensitivity are higher in chronic than episodic migraine patients (Bigal et al., 2008). Cutaneous allodynia has thus been suggested as a risk factor for disease progression (Bigal et al., 2008) or no remission (Manack et al., 2011). Therefore, assessing cutaneous sensitivity in animals in response to known migraine triggers has been proposed as a useful approach to modeling migraine (Boyer et al., 2014; Oshinsky and Gomonchareonsiri, 2007; Pradhan et al., 2014). For instance, in rats, dural stimulation with an inflammatory soup induces both cephalic and extracephalic allodynias (Boyer et al., 2014; Edelmayer et al., 2009; Oshinsky and Gomonchareonsiri, 2007), that is associated with trigeminospinal central sensitization (Boyer et al., 2014) and impaired descending inhibitory (Boyer et al., 2014) and/or facilitatory (Edelmayer et al., 2009) pain controls.

Systemic administration of nitroglycerin (NTG) is an extensively used model of migraine in both human and animals (Ashina et al., 2013). In healthy subjects, systemic NTG triggers a headache. In migraineurs, it induces, in addition, a delayed headache with migraine features (Ashina et al., 2013). Such NTG-induced migraine-like headache is associated with thermal cephalic allodynia (de Tommaso et al., 2004). In rodents, systemic NTG produces extracephalic both thermal and mechanical allodynia (Bates et al., 2010; Brennan et al., 2013; Ferrari et al., 2016; Pradhan et al., 2014; Tassorelli et al., 2003), as well as hyperalgesia at the hindpaw formalin test (Greco et al., 2015; Tassorelli et al., 2003). However, these results are not in agreement with clinical studies reporting that migraine headache

*Corresponding author at: INSERM/UCA U1107, Neuro-Dol: Douleur Trigéminale et Migraine, Faculté de Chirurgie Dentaire, 2 rue de Braga, 63100 Clermont-Ferrand, France. Fax: +33-4-73-17-73-06.
E-mail address: radhouane.dallel@udamail.fr (R. Dallel).

Abbreviations: BSA, bovine serum albumin; CGRP, Calcitonin gene-related peptide; ERK, extracellular signal-regulated kinases; ISDN, isosorbide dinitrate; MDH, medullary dorsal horn; NHS, normal horse serum; NTG, nitroglycerin; RM ANOVA, repeated measures analysis of variance; TBS, Tris-buffered saline; TX, Triton X; WDR, wide dynamic range.

http://dx.doi.org/10.1016/j.neuroscience.2016.12.028
0306-4522/

does not typically produce cutaneous hypersensitivity below the C8 dermatome (Ashkenazi et al., 2007). Surprisingly, though, only few studies have examined the effect of systemic administration of nitric oxide donors on cephalic cutaneous sensitivity. Moreover, they provided conflicting results. One study performed on only three restrained rats reported that systemic administration of NTG decreases the periorbital von Frey thresholds (Oshinsky and Gomonchareonsiri, 2007). Two other studies in mice found either a decrease (Farkas et al., 2016) or no change (Kaufmann et al., 2015) in the periorbital von Frey thresholds after systemic NTG. Similarly, either an increase in rats (Greco et al., 2015) or no change in mice (Grigoraay et al., 2013) in the duration of the face-rubbing behavior was found after formalin injection into the upper lip in rats that had been pre-treated with NTG. Finally, two studies found that NTG administration raises the duration of the face-rubbing behavior induced by the subcutaneous injection of Calcitonin gene-related peptide (CGRP) into the upper lip (Capuano et al., 2014; Yao and Sessle, 2008). Therefore, combining behavioral, immunohistochemical, and *in vivo* electrophysiological approaches, this study investigated the effect of systemic administration of the nitric oxide donor, isosorbide dinitrate (ISDN), on cephalic and extracephalic cutaneous sensitivities and on the medullary dorsal horn (MDH) neuronal activation. ISDN was selected because: (i) it reliably produces headache in migraineurs but less often in healthy subjects (Bellantonio et al., 1997; Castellano et al., 1998), (ii) it is less hypotensive than NTG (Manabe et al., 2001), (iii) it has an injectable form, so the same solution can be injected in humans as well as animals, allowing comparison between preclinical and clinical results, and (iv) it does not need to be dissolved in alcohol and propylene glycol, thereby reducing non-specific effects (Olesen and Jansen-Olesen, 2012).

EXPERIMENTAL PROCEDURES

Animals

Animal experiments were performed according to the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983), the Directive 2010/63/UE of the European Parliament and the Council on the protection of animals used for scientific purpose. Protocols for animal care and use applied in this work were approved by the appropriate local committee at the University of Clermont-Ferrand-Auvergne (no. CE 28-12).

Adult male Sprague–Dawley rats (250–275 g; Charles River, L'Arbresle, France) were raised at 23 ± 1 °C in plastic cages (3–4 rats per cage; held in Iffa-Credo units) with soft bedding and water and food *ad libitum* in a 12-h/12-h dark/light cycle for at least one week before experiment. All efforts were made to minimize the number of animals used. Numbers of animals were selected according to previous experience (Boyer et al., 2014; Lapirot et al., 2011; Miraucourt et al., 2009; Raboisson and Dallel, 2004), that is a balance between usual sample sizes in the field and the need to reduce the use of animals in pain experiments. Experiments were performed on 74 animals (6–8 rats/group). All experimenters were blind to treatment conditions. Rats were randomized into treatment groups before any assessment.

BEHAVIORAL TESTING

Habituation

Animals were tested in light conditions, between 11:00 and 19:00 h, in a quiet room, at 23–24 °C. Test boxes (30 × 30 × 30 cm) had three mirrored sides. Animals were habituated for 2 days in the test environment. During each day, animals were first placed in this test box for 30-min to minimize stress. They were then gently restrained and the experimenter simulated an injection into the vibrissae area or the hindpaw, using a similar syringe as that in the real test. During behavioral tests, rats had access to neither water nor food. Each rat was used only once. At the end of the experiment, the rat was killed by intraperitoneal (i.p.) injection of a lethal dose of urethane (> 1.5 g/kg) and death was confirmed by permanent cessation of cardio-respiratory functions.

Face formalin test

Animals were randomly included into four groups (6–8 rats/group). Rats were injected with formalin (50 μl, 1% into the right vibrissa pad; Raboisson and Dallel, 2004) and then placed into the test box for a 45-min period. This observation period was divided into 15 3-min blocks. We computed a nociceptive score for each block: the time (in seconds) the animal spent rubbing the vibrissa pad with only the forepaw. The rubbing behavior was recorded using a video camera. Formalin was injected either 2 h after i.p. administration of saline or 1, 2 or 4 h after i.p. administration of isosorbide dinitrate (ISDN, 10 mg/kg, Sanofi-aventis, France).

Hindpaw formalin test

Rats were randomly included into two groups (eight rats/group) and received formalin (50 μl, 1%) into the right plantar hindpaw (Abbott et al., 1995). After injection, animals were placed into the test box for a 45-min period. This observation period was divided into 15 3-min blocks. We computed a nociceptive score for each block: the time (in seconds), the animal spent licking the right hindpaw. The licking behavior was recorded using a video camera. Formalin was injected 2 h after intraperitoneal administration of either saline or ISDN (10 mg/kg).

IMMUNOCYTOCHEMISTRY

MDH activation was studied using phospho-extracellular signal-regulated kinase (ERK) expression. Rats were randomly included into two groups (eight rats/group) and received formalin (50 μl, 1%) into the right vibrissa pad 2 h after i.p. administration of either saline or ISDN (10 mg/kg). Five min after formalin injection rats were anesthetized with urethane (1.5 g/kg, i.p.) and perfused transcardially with heparinized saline (25 IU heparin/mL, at 37 °C) and then phosphate-buffered solution (0.1 M,