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Differential trigeminovascular nociceptive responses in the thalamus in the familial hemiplegic migraine 1 knock-in mouse: A Fos protein study

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ABSTRACT

Familial hemiplegic migraine type 1 (FHM-1) is a monogenic subtype of migraine with aura caused by missense mutations in the *CACNA1A* gene, which encodes the pore-forming α1 subunit of voltage-gated neuronal $Ca_V2.1$ (P/Q-type) calcium channels. Transgenic knock-in mice expressing the *CACNA1A* R192Q mutation that causes FHM-1 in patients show a greater susceptibility to cortical spreading depression, the likely underlying mechanism of typical human migraine aura. The aim of this study was to compare neuronal activation within the trigeminal pain pathways in response to nociceptive trigeminovascular stimulation in wild-type and R192Q knock-in mice. After sham surgery or electrical stimulation of the superior sagittal sinus for 2 h, or stimulation preceded by treatment with naratriptan, mice underwent intracardiac perfusion, and the brain, including the brainstem, was removed. Fos expression was measured in the trigeminocervical complex (TCC) and the lateral (ventroposteromedial, ventrolateral), medial (parafascicular, centromedian) and posterior thalamic nuclei. In the TCC of wild-type animals, the number of Fos-positive cells increased significantly following dural stimulation compared to the sham control group ($P < 0.001$) and decreased after naratriptan treatment ($P < 0.05$). In R192Q knock-in mice, there was no significant difference between the stimulated and sham ($P = 0.10$) or naratriptan pre-treated groups ($P = 0.15$). The number of Fos-positive cells in the R192Q stimulated group was significantly lower compared to the wild-type stimulated mice ($P < 0.05$). In the thalamus, R192Q mice tended to be more sensitive to stimulation compared to the sham control in the medial and posterior nuclei, and between the two strains of stimulated animals there was a significant difference in the centromedian ($P < 0.005$), and posterior nuclei ($P < 0.05$). The present study suggests that the FHM-1 mutation affects more rostral brain structures in this experimental paradigm, which offers a novel perspective on possible differential effects of mutations causing migraine in terms of phenotype-genotype correlations.

Introduction

Familial hemiplegic migraine (FHM) is a rare monogenic subtype of migraine characterized by hemiplegic aura (Headache Classification Committee of The International Headache Society, 2004). Two-thirds of FHM patients also experience 'typical migraine attacks' (Thomsen et al., 2002), which raises the question of the relationship between the mutation and more common forms of migraine. FHM-1 is caused by missense mutations in the *CACNA1A* gene (Ophoff et al., 1996), which encodes for a subunit of $Ca_V2.1$ channels, that are located on presynaptic terminals and involved in controlling transmitter release (Mintz et al., 1995; Westenbroek et al., 1995). $Ca_V2.1$ channels are expressed throughout the brain, including regions implicated in migraine, such as the trigeminocervical complex, brainstem and cortex (Goadsby et al., 2009). Blockade of $Ca_V2.1$ channels has been shown to prevent pre-synaptic calcitonin gene-related peptide (CGRP) release from perivascular trigeminal sensory nerve fibers (Akerman et al., 2003) and can have a role in trigeminocervical second order neurotransmission (Shields et al., 2005).

Functional consequences of FHM-1 mutations on single-channel $Ca_V2.1$ properties have been investigated by overexpressing wild-type and mutant *CACNA1A* cDNA in neuronal cultures of *Cacna1a* knock-in mice (Tottene et al., 2002), suggesting that FHM-1 mutations, including

Abbreviations: CGRP, calcitonin gene-related peptide; CSD, cortical spreading depression; CM, centromedian thalamic nucleus; FHM, familial hemiplegic migraine; PF, parafascicular thalamic nucleus; Po, Posterior thalamic nucleus; TCC, trigeminocervical complex; TNC, Trigeminal nucleus caudalis; VPL, ventroposterolateral thalamic nucleus; VPM, ventroposteromedial thalamic nucleus.

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the R192Q mutation, exhibit "gain-of-function" properties, although there have been studies that suggest differently (Cao and Tsien, 2005; Cao et al., 2004). *In vivo*, R192Q knock-in mice exhibit an increased calcium influx through $Ca_V2.1$ channels and increased susceptibility to cortical spreading depression (CSD) (Eikermann-Haerter et al., 2011; van den Maagdenberg et al., 2004), the probable underlying mechanism of typical human aura (Hadjikhani et al., 2001), seen in experimental animals. Increased channel availability, increased calcium influx at lower membrane potentials and increased probability of cortical glutamate release in R192Q knock-in mice were shown to explain the increased susceptibility to CSD (Tottene et al., 2009).

Activation, or the perception of activation, of the trigeminal system innervating the cranial vasculature is thought to be involved in the pain of migraine (Goadsby et al., 2002). Trigeminovascular stimulation in animals activates neurons in the trigeminocervical complex (TCC) and at higher pain processing centers (Benjamin et al., 2004; Hoskin et al., 1999, 2001; Kaube et al., 1993; Zagami and Lambert, 1990). Functional imaging studies consistently show thalamic activation following experimental trigeminal nociceptive stimulation and during migraine attacks (DaSilva et al., 2007; Sprenger and Goadsby, 2010). Given the prominent role of FHM mutations in modulating the susceptibility to CSD, the effects of the mutation on activation of the trigeminovascular system and on central nociceptive processing pathways are of interest.

In the current study we used electrical stimulation of the superior sagittal sinus, which has been previously shown to cause activation of a number of nuclei involved in trigeminovascular nociceptive activation in experimental animals (Andreou et al., 2010b; Bergerot et al., 2006). We used the identification of the protein product of the immediate early gene *c-fos*, as a marker of cellular activation, which is rapidly and transiently expressed in response to neuronal stimulation (Morgan and Curran, 1989). Our hypothesis was that trigeminovascular activation might differ in R192Q knock-in compared to wild-type animals, and the difference might be an enhanced response to stimulation. The data have been presented in preliminary form (American Headache Society, Los Angeles 25–27 June, 2010, Park et al., 2010).

Materials and methods

Animal preparation

Experiments were conducted after ethical review by the Institutional Animal Care and Use Committee at the University of California, San Francisco or under the UK Home Office Animals (Scientific Procedures) Act (1986). We used homozygous R192Q knock-in mice sex-matched between groups with the human R192Q (FHM-1) missense mutation in the *CACNA1A* gene ($n = 48$, 20–30 g) raised in a mixed 129 C57BL/6Jico background (van den Maagdenberg et al., 2004), and non-transgenic littermates were used for comparison ($n = 41$, 20–30 g). All mice were bred at the Leiden University Medical Center and transported to our laboratory. Mice were initially anesthetized with sodium pentobarbitone (80 mg kg^{-1}; i.p.) and further anesthesia was maintained with i.p. bolus injections of sodium pentobarbitone (40 mg kg^{-1}). Level of anesthesia was assessed by the absence of paw withdrawal. The trachea was cannulated to maintain an open airway and the temperature was maintained between 36.5 °C and 37.5 °C throughout (Homeothermic blanket system for rodents, Harvard Instruments, Kent, U.K.). The mice were placed in a stereotaxic frame; the skull was drilled around the superior sagittal sinus, and the bone was gently removed to expose the superior sagittal sinus (SSS). The area was bathed in mineral oil and the animals rested for 1 h subsequent to further manipulation, to minimize non-specific Fos protein expression (Hoskin and Goadsby, 1999). Two platinum hook electrodes were placed on dura mater over the SSS and effort was taken to minimize contact between the cortex and stimulating electrodes to reduce the risk of current spread to the cortex (Grass Instruments S88 Stimulator, West Warwick, RI, USA). The animals were then separated into four groups as follows:

1. Surgical controls— animals received all surgery followed by transcardiac perfusion (wild-type (WT): $n = 11$; R192Q: $n = 14$).
2. Sham control — after the one hour rest period the hook electrodes were placed on the SSS and the animals remained in the frame for a further 2 h, receiving no stimulation or any other manipulation (WT: $n = 10$; R192Q: $n = 13$).
3. Stimulation — after one hour rest period the stimulating electrodes were placed on the SSS and animals received 2 h of stimulation (0.5 Hz, 0.5 ms duration at 400 μA). Fifteen minutes prior to stimulation mice received 0.05 ml i.p. bolus of saline (WT: $n = 10$; R192Q: $n = 11$).
4. Stimulation with naratriptan (10 mg kg^{-1}, i.p.) — after the one hour rest period the stimulating electrodes were placed on the SSS and animals received 2 h of stimulation (0.5 Hz, 0.5 ms duration at 400 μA). Fifteen minutes prior to stimulation mice received an i.p. bolus of naratriptan (WT: $n = 10$; R192Q: $n = 10$).

Following the experiment, animals were euthanized by an overdose of pentobarbitone (200 mg/kg, i.p.). The animals were then perfused via the ascending aorta with 0.9% saline (50 ml), followed by 4% paraformaldehyde (50 ml) in 0.1 M phosphate buffer saline (PBS; pH 7.4). The brain and spinal cord were removed and stored overnight in the same fixative, and then placed in a cryoprotectant solution (20% sucrose, 30% ethylene glycol in 0.1 M PBS) for 48 h before sectioning. The brain and upper cervical spinal cords were sectioned serially on a freezing cryostat. Sections (30 μm) that spanned the trigeminocervical complex (trigeminal nucleus caudalis, cervical spinal cord levels C_1 and C_2) and the thalamus were collected in PBS, every 300 μm.

Tissue preparation and immunohistochemistry

Tissue sections (30 μm) were processed free floating. They were placed in phosphate buffer saline (PBS-Sigma P4417-100 tbs), using twelve well plates (Corning Netwell, Fisher Scientific). Sections were washed in 50% alcohol for 30 min, then transferred for 30 min into a 3% hydrogen peroxide solution (Sigma H1009) made up with 50% alcohol. After three five minute washes in PBS, sections were incubated for 2 h in a blocking solution of 5% Normal Goat Serum (Vector,S-1000). Forty-eight hour primary incubation was followed with c-Fos antibody (sc52, rabbit polyclonal IgG, Santa Cruz), 1:7500, at 4 °C, using a diluent solution made up with PBS, bovine albumin solution (0.1%) (Sigma A9647), 2% normal goat serum, and 0.2% Triton (X100 solution-Sigma 93443). After primary incubation and three five minute washes in PB, sections were incubated for 2 h at 1:500 with secondary goat biotinylated anti-rabbit (Vector BA1000). After three five minute washes in PBS, sections were incubated for 30 min with Avidin-Biotin peroxidase Complex (Elite PK-6100 Standard Vectastain ABC kit, Vector); washed in PBS before a final DAB reaction with 3,3 diaminobenzidine tetrahydrochloride dihydrate and nickel solution (DAB substrate kit for peroxidase-SK-4100, Vector).

After a wash in water, the sections were mounted on slides, air dried, dehydrated through graded alcohol solutions and cover slipped with DPX medium (Fisher Scientific).

Immunocytochemical controls

Omission of either primary or secondary antibody abolished the immunocytochemical staining completely indicating method specificity. Fos immunoreactivity was distinguishable by cellular location: the nucleus was always labeled by an intense dark brown to black locating the Fos protein.