Please cite this article in press as: O'Brien M, Cairns BE. Monosodium glutamate alters the response properties of rat trigeminovascular neurons through activation of peripheral NMDA receptors. Neuroscience (2016), http://dx.doi.org/10.1016/j.neuroscience.2016.08.007

Neuroscience xxx (2016) xxx–xxx

MONOSODIUM GLUTAMATE ALTERS THE RESPONSE PROPERTIES OF RAT TRIGEMINOVASCULAR NEURONS THROUGH ACTIVATION OF PERIPHERAL NMDA RECEPTORS

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Abstract—Ingestion of monosodium glutamate (MSG) has been shown to cause headaches in healthy individuals and trigger migraine-like headaches in migraine sufferers. We combined immunohistochemistry, *in vivo* electrophysiology, and laser Doppler recordings of dural vasculature to investigate the effect of systemic administration of MSG on the trigeminovascular pathway. Immunohistochemical analysis confirmed the expression of NMDA receptors on nerve fibers innervating dural blood vessels and excitatory amino acid transporter 2 on dural blood vessels. Systemic administration of MSG (50 mg/kg) evoked an increase in ongoing discharge in 5/6 spinal trigeminal subnucleus caudalis (SpVc) neurons with dural input recorded from male and female rats, respectively, as well as lowering their mechanical activation threshold. There were no sex-related differences in these effects of MSG. Neuronal discharge and mechanical sensitization were significantly attenuated by co-injection with the peripherally restricted NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid in both sexes. Systemic administration of MSG induced a 24.5% and 20.6% increase in dural flux in male and female rats, respectively. These results suggest that MSG-induced headache is mediated by the activation of peripheral NMDA receptors and subsequent dural vasodilation. Peripheral NMDA receptors are a potential target for the development of new drugs to treat headaches.

Key words: Doppler flowmetry, dura, nerve fibers, sensory neuron, trigeminal.

INTRODUCTION

The pathophysiology of migraine has yet to be fully elucidated, however, the excitation of trigeminal afferent fibers which innervate the dura and converge at the spinal trigeminal subnucleus caudalis (SpVc), has been identified as a key mechanism in headache pain (Burstein et al., 2015). Historically, migraine has been considered a vascular headache, largely based on the observation of dilation of retinal and temporal blood vessels during a migraine attack (Goltman, 1936; Wolff et al., 1953). Recent studies are conflicting in their support of a vascular mechanism in migraine attacks (Friberg et al., 1991; Zwetsloot et al., 1993; Kruuse et al., 2003; Asghar et al., 2010). Dural blood vessels are heavily innervated by trigeminal afferent fibers, however, and several scenarios exist which could lead to excitation of afferents and also vasodilation during a migraine attack. In the first scenario, dilation of the highly innervated dural blood vessels can activate trigeminovascular afferent fibers, potentially leading to the sensitization of the trigeminovascular system (Levy and Burstein, 2011). In the second scenario, dural afferent fibers which have already been activated may induce vasodilation via neurogenic inflammation by releasing calcitonin gene related peptide (CGRP) and substance P (SP) in Levy and Burstein (2011).

Glutamate, an excitatory neurotransmitter, has been linked to migraine pathophysiology for several reasons. Glutamate levels in blood plasma, platelets, and cerebrospinal fluid (CSF) are elevated in migraineurs long after a migraine attack (Martinez et al., 1993; Cananzi et al., 1995; Eufemia et al., 1997), and several genetic variants affecting glutaminergic neurotransmission have been identified in migraine sufferers (Schürks, 2012; Burstein et al., 2015). Glutamate is also well known to be involved in the sensitization of trigeminal afferent fibers (Cairns et al., 2007; Gazerani et al., 2010b; Laursen et al., 2014), as well as the transduction of nociceptive signaling (Klafke et al., 2012; Chan and MaassenVanDenBrink, 2014). Monosodium glutamate (MSG) is a naturally occurring form of glutamic acid, and is an International Headache Society recognized trigger for headache. MSG-related headache is classified as mild to moderate in non-migraineurs, but classified as episodic migraine in those who suffer from migraine (Headache Classification Committee of the International Headache Society, 2013). In recent studies, a single oral

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Abbreviations: ANOVA, analysis of variance; BBB, blood–brain barrier; CGRP, calcitonin gene related peptide; EAAT1-3, excitatory amino acid transporters 1-3; LSD, least significant difference; MMA, middle meningeal artery; MSG, monosodium glutamate; MT, mechanical threshold; WDR, wide-dynamic range.

http://dx.doi.org/10.1016/j.neuroscience.2016.08.007

dose of 150 mg/kg taken consecutively for five days resulted in headache and muscle tenderness when given to healthy young volunteers (Baad-Hansen et al., 2010; Shimada et al., 2013, 2015), which merit further studies as to the mechanism of MSG.

It is unknown how elevated levels of glutamate contribute to headache. Because glutamate does not cross the blood–brain barrier (BBB) (Gasparini and Griffiths, 2013), ingestion of MSG likely induces headache through a peripheral mechanism. To investigate the potential mechanisms by which MSG may induce headache, we assessed (i) the expression the NMDA receptor and glutamate transporters in the dura, as well as (ii) the neuronal and (iii) vascular effects of systemic administration of MSG in rats. We also assessed whether co-administration of a peripherally restricted NMDA receptor antagonist, (2R)-amino-5-phosphonovaleric (APV), could attenuate effects of MSG administration on SpVc neurons.

EXPERIMENTAL PROCEDURES

Animals

Male (305–480 g, $n = 30$) and female (245–330 g, $n = 28$) Sprague–Dawley rats (Charles River, Canada) were used for these experiments. Animals were housed in groups of two or three and were subject to a 12-h light/dark cycle. Food and water were available *ad libitum*. All animal procedures were reviewed and approved by the University of British Columbia Animal Care Committee.

Surgical procedure for electrophysiological and Doppler recordings

Male and female Sprague–Dawley rats were anesthetized with isoflurane (2–2.5% in oxygen 97–98%) and artificially ventilated via a trachea tube. Temperature was continually measured with a rectal thermometer and maintained at 37 ± 0.5 °C using a heating pad (Fine Science Tools, Vancouver, Canada). Blood pressure was measured via carotid artery cannulation. The heart rate, blood pressure, and core body temperature were monitored throughout the experiments. The femoral vein was cannulated to administer drugs. Animals were placed in a Kopf stereotaxic frame, and the skull was exposed.

For electrophysiology experiments, the right parietal and the frontal bone were removed from the lambdoid suture to anterior of the orbit. An incision was also made in the skin and muscle over the neck to expose the brainstem, the dura was removed and a C1 laminectomy was performed. The exposed brainstem was bathed in mineral oil.

A separate group of animals were used for dural blood flow experiments. The closed window method was used to measure dural blood flux with a Doppler flowmeter (Akerman et al., 2013). Briefly, the parietal bone was thinned over the middle meningeal artery (MMA) using a small drill, creating a ''closed window". The Doppler probe was held in place over the window by a cotton-tipped applicator lowered in position by a micro manipulator. Mineral oil was used to keep the cotton-tipped applicator and exposed bone moist.

Electrophysiological recordings

Extracellular action potentials of SpVc neurons with dural receptive fields were recorded with a parylene-coated tungsten microelectrode (0.010", 2 MΩ, A-M Systems Inc., Carlsborg, WA, USA) in male ($n = 21$) and female ($n = 19$) rats. The recording electrode was lowered into the exposed brainstem over the spinal subnucleus caudalis (SpVc), 1–2 mm lateral and 1–2 mm caudal to the obex and at depth ranging from 50 to 2800 μm at a 33° angle. The V1 and V2 dermatomes of the skin were brushed while the electrode was lowered. To identify potential areas of the SpVc which may have dural input, mechanoreceptors innervating the dura were identified by their response to mechanical stimulation applied to the dura. Mechanoreceptors innervating the dura were identified by their response to mechanical stimulation applied to the dura. An electronic von Frey hair (model 1601C, Life Science, USA) was used to assess the mechanical activation threshold for dural responsive SpVc neurons. In some experiments multiple neurons, which could be differentiated by their action potential characteristic, shared a dural receptive field; in those cases, the acquired data from both neurons were used and spike sorting was applied with Spike 2 software (Cambridge Electronics, UK). The neurons were characterized by their receptive fields, their response to innocuous (brush) and noxious (pinch) stimuli applied to the skin, and also the latency to action potential discharge following electrical stimulation of the dura (0.5–5 mA, 0.5 ms, 0.5 Hz). In addition, the response of neurons to direct mechanical stimulation of the temporalis muscle with a blunt probe was determined by temporarily pulling the skin away from the muscle.

Following the identification of a SpVc neuron with a dural receptive field, the baseline mechanical threshold (MT) was measured. Briefly, the von Frey hair was pressed against the dural receptive field with increasing force until it elicited an action potential discharge; the smallest force which elicited an action potential was recorded as the MT. The mean baseline MT was calculated from five evoked responses separated by one-minute intervals. A five-minute baseline was recorded to assess ongoing discharge in the neuron, after which 50-mg MSG was administered alone or in combination with APV (5 mg/kg or 50 mg/kg). Post-injection discharge was monitored for 5 min. Cumulative discharge was calculated by subtracting the number of action potentials during the pre-injection period from those during the post-injection period. The criterion for neuronal activation was a positive difference between the total number of action potentials fired following MSG administration and action potentials fired during the baseline period. The MT was then assessed for a period of 10 min. Mean MT values were calculated for the two 5-min post-injection time periods. Animals were euthanized at the end of the experiments (pentobarbital 100 mg/kg).

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