NEUROCHEMICAL CHANGES FOLLOWING ACTIVATION OF THE DURAL AFFERENT

By

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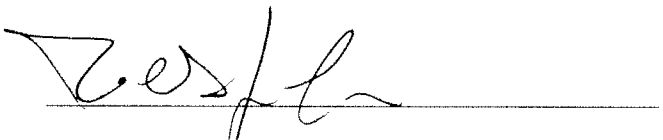

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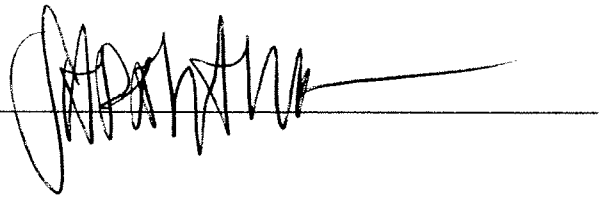
Signed: 

Abstract

The objective of the study was to explore the neurochemical effects of dural afferent activation in different regions of the brain, the ventral tegmental area (VTA) and the nucleus accumbens (NAcc), and determine how these mechanisms play a role in pain relief following onset of migraine pain. Inflammatory mediators (IM) or synthetic interstitial fluid (SIF) were injected into the dura of rats to initiate dural afferent activation, followed by treatments of saline (Sal) or lidocaine (Lido) injection into the rostral ventromedial medulla (RVM). Upon collection of VTA and NAcc tissue sections from each treatment group, cells were immunolabeled for FOS to determine action potential prevalence associated with dopamine release. VTA showed increased FOS expression in rats treated with IM + Sal, while NAcc showed greater expression in IM + Lido treatment group. These effects may be characteristic of the role of lidocaine within the reward system, although signaling within the dopaminergic system may be altered upon projection into the NAcc region. These findings are consistent with the possibility of outside factors of signal propagation throughout the dopaminergic system. Further research on the underlying mechanisms of the dopaminergic system may provide novel treatment strategies of migraine pain relief.

Introduction

While theories of neurochemical changes occurring in migraine pain have largely advanced with new imaging technologies of obtaining data in various brain regions, the mechanisms of signal propagation within the dopaminergic system are not yet fully understood. The VTA and NAcc are two central components within this "brain reward system," named for the increase in dopamine transmission levels associated with rewarding activity. Given the knowledge that the dopaminergic mesolimbic pathway originates in the VTA and projects to the NAcc, dopaminergic input via the VTA is believed to indirectly induce the activity of neurons within the NAcc, causing dopamine release.

Here, IM is administered to activate the dural afferent, followed by treatment with lidocaine, to characterize the pathways involved in migraine headache and subsequent pain relief. Lidocaine, a local anesthetic that has been tested for effectiveness as an intranasal treatment of acute migraine, provides pain relief by preventing pain signal propagation to the brain. By blocking voltage gated sodium channels, the postsynaptic neuron fails to depolarize and transmit an action potential. Lidocaine is most commonly used as an injectable local anesthetic for minor surgical procedures, in topical ointments for skin irritation, and as an antiarrhythmic agent. As an immediate pain reliever it is thought to play a pivotal role in activation of the reward system of the brain, likely triggering release of dopamine after onset of inflammation.

By exploring the avenues through which migraine pain can be treated, more effective treatments involving alteration of the dopaminergic system may be administered.

Materials and Methods

Animals

Male, Sprague-Dawley rats (250–300 g; Harlan) were maintained on a 12-hr light/dark cycle with food and water ad libitum. All procedures were performed according to the policies and recommendations of the IASP, the NIH guidelines for laboratory animals, and by the IACUC recommendations of the University of Arizona and Oregon Health and Science University.

Surgical Preparation

Dura cannulation

Anesthesia was induced with ketamine/xylazine (80 mg/kg and 12 mg/kg i.p., respectively). Rats were placed in a stereotactic headholder and a 2 cm incision was made to expose the skull. A 1 mm hole (1 mm left of midline, 1 mm anterior to bregma) was made with a hand drill (DH-0 Pin Vise, Plastics One Inc., Roanoke, VA) to carefully expose the dura. A guide cannula (22 GA, #C313G, Plastics One Inc.), designed to extend 0.5 mm from the pedestal to avoid irritation of the dural tissue, was inserted into the hole and sealed into place with glue. Two additional 1 mm holes were made caudal to the cannula to receive stainless steel screws (#MPX-080-3F-1M, Small Parts Inc., Miami Lakes, FL) and dental acrylic was used to fix the cannula to the screws. A dummy cannula (#C313DC, Plastics One Inc.) was inserted to ensure patency of the guide cannula, the skin was sutured closed around the dried acrylic and Amikacin C (5 mg/kg, i.m.) was administered. Rats were housed separately and allowed 6–8 days recovery. Cannula placement and integrity of the dura was confirmed with microinjection of 10 μL of India ink, which spread 3–5 mm on the dorsal aspect of the dura and did not penetrate to the brain.

RVM cannulation

Some rats also received an additional bilateral guide cannula (26GA, #C235G-1.2mm, Plastics One Inc.) directed to the RVM. The cannula was placed at: −11.0 mm from bregma, −7.5 mm from the dura and 0.6 mm on either side of the midline. Injections were made by expelling 0.5 μL through an injection cannula protruding 1 mm beyond the tip of the guide. Cannula placement was confirmed with India ink and microscopic examination of Nissl-stained medullary sections. Acute single injections into the RVM were performed by inserting a 30 gauge needle attached to a Hamilton syringe and expelling 0.5 μL at the same coordinates.

Dural Inflammation

Inflammation of the dura was produced by injecting 10 μL of a cocktail of inflammatory mediators through an injection cannula (28GA, #C313I, Plastics One Inc.) cut to fit the guide cannula. Composition of synthetic interstitial fluid (SIF) and inflammatory mediators (IM) was modified from previous reports[4,5]. The SIF consisted of 10 mM Hepes, 5 mM KCl, 1 mM $MgCl_2$, 5 mM $CaCl_2$, and 135 mM NaCl, pH 7.3. The IM solution was formulated with 2 mM histamine, serotonin, bradykinin and 0.2 mM PGE_2 in 10 mM Hepes buffer, pH 5.0, representing twice that used by Burstein and colleagues[4,5]. Pilot studies were performed with multiples of these concentrations, and the formulation providing a robust and consistent, yet submaximal, response was employed.

Experimental Protocols

Baseline behavioral responses to probing of the face and hindpaws were obtained from all rats prior to drug administration. Rats then received either SIF or IM, and behavioral

responses were determined at 1 hr intervals for 6 hrs. Drug administration was performed either 10 min prior to dural inflammation or at time points after inflammation by either systemic injection or RVM microinjection. Sumatriptan succinate (GlaxoSmithKline), naproxen (Sigma), L-732,138 (NK-1 antagonist, Tocris), α-CGRP$_{(8–37)}$ (CGRP-antagonist, Bachem), YMO22 (CCK2-antagonist, Tocris), CCK-8(s) (American Peptide Inc.), bupivacaine HCl (Sigma), dermorphin-saporin conjugate and saporin alone (Advanced Targeting Systems) were used. Doses used were kept within published literature.

Immunohistochemistry Protocol

Rats were used for determination of FOS expression. Animals were anesthetized with ketamine/xylazine, perfused with phosphate-buffered saline (PBS; pH 7.4) and fixed by perfusion with 500 ml of 4% paraformaldehyde in PBS using standard immunohistochemical methods. Sections 20 µm thick were cut from the VTA and NAcc and immunolabeled for FOS with standard methods. Images were acquired with a digital camera and analyzed with MetaMorph imaging software (Molecular Devices, Downington, PA). A total of 12 sections (6 VTA and 6 NAcc) were obtained from each rat, and 4 rats were used for each treatment group.

Results

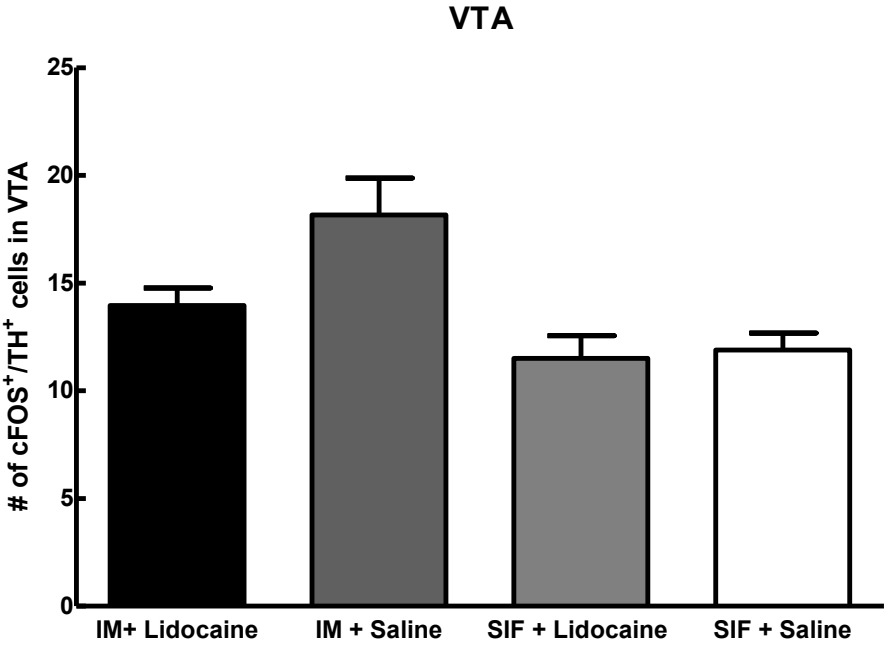


Fig 1. The graph shows average counts for cells in VTA showing FOS and tyrosine hydroxylase (TH) expression for each treatment group. Treatment group IM + Sal shows a significant increase in FOS immunolabeled cells within VTA, with all other groups markedly lower in comparison.

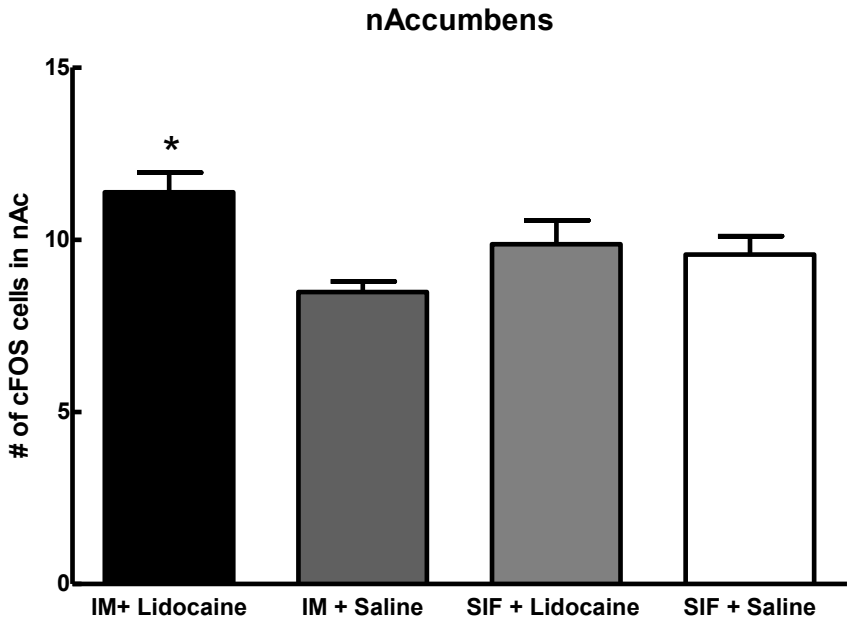


Fig 2. The graph shows average counts for cells in NAcc showing FOS and TH expression for each treatment group. Treatment group IM + Lido shows a small increase in FOS expression in NAcc compared to other groups, closely alongside SIF + Lido treatment group.

Discussion

Although lidocaine administration following onset of IM was expected to show dopaminergic activation in VTA along with NAcc, analysis of data showed that while this was the case in the NAcc, saline showed much greater dopaminergic activation in VTA. Possible explanations for high FOS expression in VTA upon injection of saline rather than lidocaine may be attributed to unknown factors involving input within VTA following dural afferent activation.

Dopamine release following lidocaine injection was expected in the NAcc as a result of input from the VTA. However, the opposing effects of lidocaine from VTA to NAcc are reminiscent of outside factors of signal propagation throughout the dopaminergic mesolimbic pathway. The mechanisms by which the onset of migraine headache and subsequent pain relief interact with the reward system are largely unknown, and the role of the VTA in providing input to the NAcc and other brain regions may be affected by a combination of interconnection inputs throughout all brain signaling pathways. The possibility of underlying modes of neurochemical input can open the avenues to greater research in the field of migraine headache and pain relief.

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