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TRIGEMINAL BRAINSTEM MODULATION OF PERSISTENT ORBICULARIS OCULI MUSCLE ACTIVITY IN A RAT MODEL OF DRY EYE

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Abstract—Altered corneal reflex activity is a common feature of dry eye disease (DE). Trigeminal sensory nerves supply the ocular surface and terminate at the trigeminal interpolaris/caudalis (ViVc) transition and spinomedullary (VcC1) regions. Although both regions contribute to corneal reflexes, their role under dry eye conditions is not well defined. This study assessed the influence of local inhibitory and excitatory amino acid neurotransmission at the ViVc transition and VcC1 regions on hypertonic saline (HS) evoked orbicularis oculi muscle activity (OOemg) in urethane-anesthetized male rats after exorbital gland removal (DE). HS increased the magnitude of long-duration OOemg activity (OOemgL, >200 ms) in DE compared to sham rats, while short-duration OOemg activity (OOemgS, <200 ms) was similar for both groups. Inhibition of the ViVc transition by muscimol, a $GABA_A$ receptor agonist, greatly reduced HS-evoked OOemgL activity in DE rats, whereas injections at the VcC1 region had only minor effects in both groups. Blockade of $GABA_A$ receptors by bicuculline methiodide at the ViVc transition or VcC1 region increased HS-evoked OOemgL activity in DE rats. Blockade of N-methyl-D-aspartate (NMDA) receptors at either region reduced HS-evoked OOemgL activity in DE and sham rats. GABA$\alpha\beta$3 receptor density was reduced at the ViVc transition, while NMDA receptor density was increased at both regions in DE rats. Loss of GABAergic inhibition at the ViVc transition coupled with enhanced NMDA excitatory amino acid neurotransmission at the ViVc transition and the VcC1 region likely contribute to altered corneal reflexes under dry eye conditions. Published by Elsevier Ltd on behalf of IBRO.

Key words: corneal reflex, dry eye, electromyography, orbicularis oculi, GABA receptor, NMDA receptor.

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Abbreviations: AP5, 2-amino-5-phosphonopentoic acid; AUC, area under the curve; BMI, bicuculline methiodide; DE, dry eye; GABA, gamma-aminobutyric acid; HS, hypertonic saline; MAP, mean arterial pressure; NMDA, N-methyl-D-aspartate; OOemg, orbicularis oculi muscle electromyography; OOemgL, long-duration OOemg; OOemgS, short-duration OOemg; ViVc, trigeminal subnucleus interpolaris/caudalis transition; VcC1, trigeminal subnucleus caudalis/upper cervical spinal cord junction.

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INTRODUCTION

Dry eye disease (DE) is defined by an unstable tear film, ocular inflammation and symptoms of discomfort (DEWS Report Definition, 2007). Relief from symptoms, which often include burning or stinging pain and foreign body sensation, is a primary reason that patients seek medical attention for DE (Begley et al., 2002; Rosenthal and Borsook, 2015; Vehof et al., 2013). However, peripheral treatments that reduce ocular inflammation (Behrens et al., 2006; Alves et al., 2013) often are inadequate in managing symptoms in moderate to severe DE (Asbell and Spiegel, 2010; Williamson et al., 2014). Furthermore, peripheral signs of tear film instability are not well correlated with symptoms (Johnson, 2009; Sullivan et al., 2014). These findings are consistent with the notion that pain sensation and altered protective reflexes in DE involve persistent changes in the brain; however, little is known about the underlying mechanisms that could account for altered symptoms in DE.

The corneal reflex pathway consists of: (i) trigeminal sensory neurons that supply the ocular surface, (ii) that terminate on trigeminal brainstem neurons in the subnucleus interpolaris/caudalis (ViVc) transition and the caudalis/upper cervical spinal cord (VcC1) region (Marfurt and Del Toro, 1987; Panneton et al., 2010) that integrate somatosensory signals, and project to (iii) orbicularis oculi (OO) motoneurons in the facial motor nucleus that drive eyelid closure (Ongerboer de Visser, 1980). Brief electrical or mechanical stimulation of the cornea in humans evokes a single OOemg peak of activity and at long latencies (Accornero et al., 1980; Mukuno et al., 1983; Cruccu et al., 1991). By contrast, brief electrical stimulation of facial skin or supraorbital nerve evokes two peaks of OOemg activity, a short latency short-duration peak (R1) and a long latency (R2) long-duration peak (Evinger et al., 1991; Pelligrini et al., 1995). Although the corneal reflex and the R2 phase of the eyeblink share similarities in terms of latency and duration, it is likely that elements of the underlying circuitry are different (Berardelli et al., 1985). Evidence from animal studies suggests that second-order neurons at the ViVc transition and VcC1 region contribute to corneal reflexes (Henriquez and Evinger, 2005, 2007; Rahman et al., 2014) and eyeblinks (Pellegrini et al., 1995).

The balance between $GABA_A$ receptor-mediated inhibitory mechanisms and NMDA receptor-mediated

excitatory mechanisms in spinal levels is altered after persistent inflammation (Woolf and Salter, 2000; von Hehn et al., 2012). Although corneal-responsive neurons at the ViVc transition and VcC1 regions are greatly influenced by $GABA_A$ and NMDA receptor activation in naïve animals (Bereiter and Bereiter, 1996; Hirata et al., 2003, 2004), it is not known if these receptor pathways are altered in a model for tear-deficient DE. Unlike most previous studies in which transient stimuli were presented, this study used persistent ocular instillation of hypertonic saline (HS) to evoke OOemg activity in sham and DE male rats. The rationale for this approach was that hyperosmolar tears is a common, yet persistent, feature of DE (Sullivan et al., 2014) and that ocular surface conditions influence spontaneous (Acosta et al., 1999) and evoked eyeblink rates in control subjects (Wu et al., 2014) and DE patients (Nakamori et al., 1997; Himebaugh et al., 2009). Previously we reported that DE rats displayed enhanced neural activity and OOemg to HS (Rahman et al., 2015); however, the role of $GABA_A$ and NMDA receptors at the ViVc transition and VcC1 region in mediating enhanced corneal reflexes is not known.

EXPERIMENTAL PROCEDURE

Animals

A total of 151 male rats (250–350 g, Sprague–Dawley, Harlan, Indianapolis, IN) were used in these experiments. Animals were housed in pairs with free access to food and water. Cages remained in climate- and light-controlled environment (25 ± 2 °C, 12:12-h light/dark cycle with light on at 7:00 AM). The animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota (USA) and conformed to the established guidelines set by The National Institutes of Health guide for the Care and the Use of Laboratory Animals (PHS Law 99-158, revised 2015). All efforts were made to minimize the number of the animals used for experiments.

Exorbital gland excision

Rats were anesthetized with isoflurane (3–5%) and under aseptic conditions, the fur anterior to the ear was shaved, and a small skin incision exposed the masseter muscle and the left exorbital gland. The gland was gently teased off the muscle and excised. The wound margins were treated with 2% xylocaine gel and the incision was closed with absorbable suture. In sham controls, the gland was exposed surgically but was not removed. Ketoprofen (25 mg/kg, ip) was given post-surgically for pain management. Rats survived for 14 days after surgery and prior to testing. Previously, we reported that corneal reflex activity in awake rats was enhanced at 14 days after gland removal consistent with a model for ocular hyperalgesia (Rahman et al., 2015).

Tear volume measurement

On the day of the experiment rats were anesthetized with urethane (1.2–1.5 g/kg, ip) and spontaneous tear volume was estimated by the increase in wet length of phenol red thread (ZONE-QUICK, Menicon INC., San Mateo, CA). The thread was placed in contact with the cornea/conjunctiva at its inferior-lateral edge and tear volume was measured over 2 min.

Orbicularis oculi muscle electromyography (OOemg)

The left femoral artery was catheterized to monitor arterial blood pressure that was maintained at 90–110 mmHg. Rats were allowed to breathe spontaneously. The wound margins were infiltrated with 2% lidocaine and body temperature was kept at 38 °C with a heating blanket. The rat was positioned in a stereotaxic frame and a small portion of the C1 vertebra was removed to expose the dorsal brainstem surface and allow access to the ViVc transition and VcC1 regions. Teflon-coated copper wires (0.12 mm diameter) were implanted in the upper and lower portions of the left OO muscle.

Experimental design

The recording session began at least 1 h after completion of all surgery. Hypertonic saline (HS, 2.5 M NaCl, pH: 7.2, 10 μl) was applied to the ocular surface by a microsyringe at 20-min intervals to evoke OOemg activity. The HS test solution remained on the eye during the 3-min sampling period and was washed out with artificial tears after each stimulus (total exposure time to each HS stimulus = 3–4 min) to prevent desensitization or possible damage to the ocular surface. In an initial series, rats received 0.15, 1 and 2.5 M NaCl in sequential order at 20-min intervals to quantify the OOemg responses at different osmolar concentrations. The test stimulus was 2.5 M NaCl, since this dose caused reliable increases in total OOemg that were significantly enhanced in DE versus sham rats (see Fig. 6, Rahman et al., 2015). The design for this series was: (1) HS test stimulus, (2) drug injection, (3) HS test at 10 min post-drug, (4) HS test at 30 min post-drug and (5) HS test at 50 min post-drug. Drugs were given without prior knowledge by the experimenter.

Drugs were microinjected slowly into the ViVc transition or VcC1 region, ipsilateral to gland removal, from a glass micropipette (40–80 μm tip diameter). Pipettes were filled with either phosphate-buffered saline (PBS), muscimol, a selective gamma aminobutyric acid subtype A ($GABA_A$) receptor agonist, bicuculline methiodide, a $GABA_A$ antagonist, or AP5, a selective N-methyl-D-aspartate (NMDA) receptor antagonist. Drugs were dissolved in saline and delivered in a concentration of 0.1 mM or 1 mM in a total volume of 0.2 μl over 2 min. The drug doses used here were similar or less than those reported to be effective in modulating nociceptive neurons in spinal dorsal horn (Zhang et al., 2001; Sokal and Chapman, 2003; Seagrove et al., 2004). Injection sites at the ViVc transition were approached at 28° off vertical and 45° off midline, and 1.5–2.0 mm below the brainstem surface and positioned at the level of the obex, a surface landmark defined by the caudal end of the fourth ventricle (Yoshida et al., 1991). Sites at the VcC1 region were approached at 43° off vertical, 60° off midline, within