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BEHAVIORAL AND NEUROPHYSIOLOGICAL CORRELATES OF NOCICEPTION IN AN ANIMAL MODEL OF PHOTOKERATITIS

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Abstract—Ocular exposure to ultraviolet irradiation (UVR) induces photokeratitis, a common environmental concern that inflames ocular tissues and causes pain. The central neural mechanisms that contribute to the sensory aspects of photokeratitis after UVR are not known. In awake male rats, ocular surface application of hypertonic saline evoked eye wipe behavior that was enhanced 2–3 days after UVR and returned to control levels by 7 days. Similarly, under isoflurane anesthesia, hypertonic saline-evoked activity of ocular neurons in superficial laminae at the trigeminal subnucleus caudalis/cervical (Vc/C1) region was enhanced 2 days, but not 7 days, after UVR. By contrast, the response of neurons at the interpolaris/caudalis (Vi/Vc) transition region to hypertonic saline was not affected by UVR. The background activity and convergent cutaneous receptive field areas of Vc/C1 or Vi/Vc neurons were not affected by UVR. Aqueous humor protein levels were elevated 2 and 7 days after UVR. UVR enhanced nociceptive behavior, after a latent period, with a time course similar to that of ocular neurons in superficial laminae at the Vc/C1 region. The Vc/C1 region plays a key role in primary hyperalgesia induced by UVR, whereas the Vi/Vc region likely mediates other aspects of ocular function.

Key words: eye wipe behavior, inflammation, ocular pain, trigeminal subnucleus caudalis, UV irradiation.

The eye is vulnerable to most forms of radiant energy. Exposure to ultraviolet irradiation (UVR) is particularly harmful and nearly all short wavelength (UVA, below 280 nm) and middle wavelength (UVB, 280–320 nm) UVR is absorbed by the cornea and other compartments of the anterior eye segment (Sliney, 2002). Solar energy is the main environmental source of UVA and UVB irradiation for most individuals; however, artificial sources from arc welding and tanning salons also contribute (Rieke, 1943; Tenkate, 1999). Photokeratitis is the major clinical condition after acute exposure to UVR and is characterized by anterior eye segment inflammation, reduced visual acuity, and burning-like pain of the ocular surface that develops slowly over several hours (Schein, 1992; Cullen, 2002). The neural basis for sensory changes in photokeratitis is not well defined. Most animal studies have focused on peripheral histological and biochemical aspects of UVR-induced damage to the anterior eye segment (Bergmanson, 1990; Doughty and Cullen, 1989; Zuclich, 1989), whereas less is known concerning the behavioral and central neural correlates of this injury.

The ocular surface is the most densely innervated tissue in the body (Rozsa and Beuerman, 1982) and trigeminal sensory neurons that supply this structure terminate mainly in two spatially discrete regions of trigeminal subnucleus caudalis (Vc), a rostral caudalis/interpolaris transition region (Vi/Vc), and more caudally at the trigeminal subnucleus caudalis/upper cervical spinal cord transition (Vc/C1) (Marfurt, 1981; Marfurt and Del Toro, 1987; Panneton and Burton, 1981). Previous recording studies, based mainly on response properties in naive rats (Hirata et al., 1999, 2000, 2004; Meng et al., 1997, 1998), have led to the proposal that neurons at the Vi/Vc transition and in the superficial laminae at the Vc/C1 region serve different aspects of trigeminal function in ocular pain (Bereiter et al., 2000). Although ocular inflammation is a common clinical problem that causes pain and can develop from a variety of conditions such as refractive surgery, infection, or trauma (Dargin and Lowenstein, 2008; Leibowitz, 2000), most animal models of ocular pain have been relied on approaches that involve direct nerve damage (see Belmonte et al., 2004; Wenk and Honda, 2003). Recently, we reported that endotoxin-induced uveitis (EIU), an animal model that mimics some of the early signs of anterior uveitis in humans, produced time-dependent changes in responsiveness of neurons in the caudal trigeminal sensory brainstem complex (Bereiter et al., 2005). However, EIU also produces fever with widespread effects on immune, endocrine, and cardiovascular systems (Romanovsky, 2004) that confound the delineation of mechanisms underlying the observed neural plasticity. In addition to serving as a model for photokeratitis, UVR offers several advantages for studies of ocular pain in general. As described for studies of cutaneous pain, UVR is non-invasive, produces localized inflammation, uses stimulus variables that can be readily controlled, and since it can be applied to humans, is well suited for translational research (Davies et al., 2005; Bishop et al., 2007 2009).

The goal of this study was to develop a rodent model for photokeratitis and determine the time course and intensity-related effects of UVR on behavioral and neurophysiological correlates of ocular nociception. The ocular surface was exposed to narrowband UV–B irradiation, and

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Abbreviations: EIU, endotoxin-induced uveitis; NS, nociceptive specific; RF, receptive field; UVR, ultraviolet irradiation; Vc, trigeminal subnucleus caudalis; Vc/C1, trigeminal subnucleus caudalis/upper cervical spinal cord transition; Vi, trigeminal subnucleus interpolaris; Vi/Vc, trigeminal subnucleus interpolaris/subnucleus transition; WDR, wide dynamic range.

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behavioral and electrophysiological responses were followed for 1 week. Hypertonic saline was used to activate ocular surface nociceptors, since it reliably evokes eye wipe behavior in animals and can be applied daily without causing persistent sensitization (Farazifard et al., 2005).

EXPERIMENTAL PROCEDURES

The animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota and conformed to the established guidelines set by The National Institutes of Health guide for the care of laboratory animals (PHS Law 99–158, revised 2002).

Animals and UVR

Male Sprague–Dawley rats (250–390 g, Harlan, Indianapolis, IN, USA) were briefly anesthetized with pentobarbital sodium (50 mg/kg ip) and narrow band (302 nm) UV–B radiation was delivered from a light source (UVP Co., Upland, CA, USA) at varying intensities (100, 200 or 300 mJ/cm^2). The intensity of stimulation was calculated as mJ/cm^2=mW/cm^2 $\times$ duration in seconds. The actual mW/cm^2 delivered by the light source was measured directly with a UV meter at the corneal surface (model UV-340, Mannix Co., New York, NY, USA). The rat's head, except for the eye, was covered with a UV opaque material. Each rat received a single exposure to UVR and survived for 2–7 days after irradiation. Rats that did not receive UVR were defined in the text as "controls."

Eye wipe behavior

The eye wipe test was performed using hypertonic saline as the inducing stimulus. This is a sensitive test for acute ocular irritation-related behavior in awake rats that is non-invasive, non-inflammatory by itself, and requires little or no conditioning (Farazifard et al., 2005). Rats were placed in a Plexiglas box for 1 h to habituate to the testing chamber. Test stimuli consisted of a single drop of NaCl (0.15, 2.5 and 5 M, 20 μl) applied to the ocular surface from a micropipette. Test solutions of NaCl were applied in ascending order of concentration at 30 min intervals. The number of eye wipes, defined as purposeful wiping of the face by the forelimb, was counted over 5 min on days 1, 2, 3, 5, and 7 after UVR. In preliminary experiments, we determined that the threshold concentration of NaCl to evoke eye wiping was $\sim$1 M and suprathreshold concentrations (5 M) could be applied daily for up to 2 weeks while evoking consistent responses with no visible signs of ocular inflammation. Controls were handled daily and tested at the same times as UVR-treated rats. Rats were tested without prior knowledge of UV treatment, and 6–8 rats were included in each group. Eye wipe behavior was assessed by ANOVA corrected for repeated measures. Significant treatment effects were further assessed by Newman–Keuls after ANOVA. In several cases (n=4–5 rats per treatment group; controls, 2 and 7 days after UVR) an aqueous humor sample was collected and total protein was measured by the Bradford assay (Pierce BCA Kit, Rockford, IL, USA) as an indicator of ocular inflammation.

Electrophysiology procedures

Rats were anesthetized initially with pentobarbital sodium (70 mg/kg ip) and catheters were positioned in the right femoral artery and jugular vein for monitoring blood pressure and drug infusion, respectively. After tracheotomy, animals were respired artificially with oxygen-enriched room air and anesthesia was maintained with isoflurane (1.2–2.0%). Rats received an infusion of the short-acting paralytic agent, gallamine triethiodide (25 mg/kg/h), at the time of neural recording. Expiratory end-tidal CO_2 (3.5–4.5%), mean arterial pressure (100–120 mm Hg), and body temperature (38 °C) were monitored continuously and kept within normal range. Animals were placed in a stereotaxic frame and portions of the C1 vertebra were removed to expose the lower brainstem and upper cervical dorsal horn. The exposed brainstem surface was bathed in warm mineral oil. Single neurons were recorded at the ventrolateral trigeminal subnucleus interpolaris/caudalis (Vi/Vc) transition and superficial laminae (laminae I–II) at the subnucleus caudalis/cervical (Vc/C1) junction regions (see Fig. 2). Neurons recorded at the Vi/Vc transition were approached at an angle of 28° off vertical and 45° off midline, and 1.5–2.5 mm below the brainstem surface. For neurons in superficial laminae at the caudal Vc/C1 junction region, the electrode was directed at an angle of 43° off vertical, 60° off midline, and the depth of recording was within 250 μm of the dorsal brainstem surface. Unit activity was recorded extracellularly using tungsten microelectrodes (9 MΩ, Frederick Haer Inc., Bowdoinham, ME, USA) and amplified, discriminated (model dis-1, BAK Electronics, Mount Airy, MD, USA), stored and analyzed offline on an Apple (G4) computer using a DAQ interface board and LabVIEW software (National Instruments, Austin, TX, USA).

Characterization of ocular-responsive units and experimental design

The encoding properties of units at the Vi/Vc transition and Vc/C1 junction regions were compared across three treatment groups: controls, 2 days after UVR, and 7 days after UVR. UVR was delivered at 300 mJ/cm^2 since this intensity reliably enhanced hypertonic saline-evoked eye wipe behavior. A single neuron was recorded from each rat. The search stimulus consisted of gently swiping a fine camelhair brush across the ocular surface (e.g., cornea surface and conjunctiva). Because all units included in this study had a receptive field (RF) on the cornea and/or the conjunctiva, for convenience, they are referred to in the text as "ocular units." Most ocular units also received convergent cutaneous input from periorbital skin and could be further classified as nociceptive specific (NS, pinch only) or wide dynamic range (WDR, pinch plus touch) based on the responses to a low force von Frey filament (1.2 g) and pinch with blunt forceps. A similar number of ocular neurons were classified as NS and WDR, based on cutaneous RF properties, in each treatment group, and were not analyzed separately. Several ocular units at Vi/Vc transition region were classified as "complex" and displayed an inhibitory cutaneous RF that was contiguous with the ocular surface or had no apparent cutaneous RF and were classified as cornea only. All units were activated by mechanical stimulation of the conjunctiva, and the threshold for this input was determined with a calibrated series of von Frey filaments applied to the palpebral conjunctiva below the lower eyelid. After establishing the ocular surface and periorbital skin RF characteristics to mechanical stimulation, NaCl was applied to ocular surface in a cumulative concentration regimen (0.15, 2.5 and 5 M, 20 μl) with an interstimulus interval of 30 min. Saline solutions were applied at 22–25 °C.

Neural recording data analyses

Neural recording data were acquired and displayed as peristimulus time histograms of spikes per 1-s bins, exported to a spreadsheet and analyzed off-line. Neural responses were analyzed as a total response magnitude (Rmag) for each stimulus period defined as the cumulative sum of spikes for contiguous bins in which the spike count exceeded the mean+2SD of the background activity (Hirata et al., 1999). The total Rmag was calculated for each stimulus period and can be thought of as equivalent to the "area under the curve." Units were defined as NaCl-responsive if the total Rmag exceeded a value of 10 after 2.5 or 5 M NaCl. The high-threshold convergent cutaneous RF area of each unit was determined with a small blunt forceps mapped onto standardized drawings of the rat face, digitized and quantified by a planimetric