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A delayed chronic pain like condition with decreased K_v channel activity in a rat model of Gulf War Illness pain syndrome

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ABSTRACT

Following their return from deployment, Gulf War (GW) veterans reported widespread joint and muscle pain at rates that far exceeded those of soldiers returning from other conflicts. It is widely believed that exposure to insecticides, repellants and nerve gas prophylactics contributed to the symptoms of Gulf War Illness (GWI), but an animal model of GW pain has been elusive. In our previous work, we observed that 4–8 weeks exposure to pyridostigmine bromide (PB), permethrin and chlorpyrifos could produce persistent alterations in the physiology of $Na_v1.9$ and K_v7 expressed in deep tissue nociceptors of the dorsal root ganglion. However, behavioral assessments from these same rats were not consistent with a delayed pain syndrome similar to that of GWI pain. In the present studies, we intensified the exposure to anticholinesterases PB and chlorpyrifos while retaining the same dosages. Animals receiving the intensified protocol for 30 days exhibited significant increases in resting for about 8 weeks after exposure. Thereafter, all measures were comparable to controls. Animals treated with intensified anticholinesterases for 60 days exhibited increased resting and reduced movement 12 weeks post-exposure. In whole cell patch studies, muscle and vascular nociceptor K_{DR} and K_v7 ion channels exhibited increased amplitude relative to controls (*e.g.*, normalized current and/or peak conductance) at 8 weeks post-exposures; however, at 12 weeks post-exposure, the amplitude of these currents was significantly decreased in muscle nociceptors. In current clamp studies, muscle nociceptors also manifested increased action potential duration, afterhyperpolarization and increased discharge to muscarinic agonists 12 weeks post-exposure. The decline in activity of muscle nociceptor K_{DR} and K_v7 channel proteins was consistent with increased nociceptor excitability and a delayed myalgia in rats exposed to GW chemicals.

1. Introduction

Many soldiers returning from the Persian Gulf War suffered from unusual complexes of headache, joint, muscle and abdominal pains associated with Gulf War Illness (GWI; Haley Syndrome 3; Haley and Kurt, 1997; Blanchard et al., 2006; Stimpson et al., 2006; Thomas et al., 2006; Haley et al., 2013; see also Gopinath et al., 2012). While it is well recognized that veterans returning from service overseas often report lingering musculoskeletal pain, the relative likelihood of joint, muscle, and/or abdominal pain were 3-fold greater in GW veterans than in those that were deployed elsewhere (Thomas et al., 2006; Kelsall et al., 2004; Stimpson et al., 2006; see also Kang et al., 2000). The appearance of these symptoms was typically delayed, but up to 25% of those stationed in the Persian Gulf exhibited GWI related pain prior to their return to the States (Kroenke et al., 1998). In the years that followed, the symptoms of GWI tended to remain the same or worsen over time (Hotopf et al., 2003).

The Research Advisory Committee on Gulf War Illness (GWI) concluded that pesticides could have contributed to the development of the symptoms of GWI (Binns et al., 2008; RAC, 2014). During the brief course of the Gulf War, soldiers were potentially exposed to 67 insecticides and repellants containing 37 distinct active ingredients (DoD Environmental Exposure Report: Pesticides, 2003; Binns et al., 2008). These included a variety of organophosphate, organochlorine, dialkylamide, carbamate and pyrethroid pesticides and repellants. Our laboratory has identified

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molecular adaptations in pain system neurons that result from exposure to GWI suspected pesticide neurotoxicants (permethrin, chlorpyrifos) and the nerve gas prophylactic, pyridostigmine bromide (PB; Jiang et al., 2013; Nutter et al., 2013; Nutter and Cooper, 2014). These chemicals have direct interactions with important proteins expressed in the peripheral pain (nociceptor) system. Permethrin acutely modifies the physiology of central and peripheral nervous system Na^+ channels that are essential for pain coding ($Na_v1.6$, $Na_v1.7$, $Na_v1.8$, $Na_v1.9$; Soderlund et al., 2002; Bradberry et al., 2005; Ray and Fry, 2006; Nutter et al., 2013; Nutter and Cooper, 2014). Chronic exposure to anticholinesterases chlorpyrifos and PB upregulate expression of muscarinic acetylcholine receptor proteins that couple to important voltage sensitive K^+ channels that control neuronal excitability (K_v7; Abou-Donia et al., 2003, 2004). Following an 8-week exposure to permethrin, chlorpyrifos and PB (rats), we were unable to demonstrate a pattern of behavior consistent with a chronic pain condition. Nevertheless, we observed upregulation of ion channel proteins $Na_v1.9$ and K_v7 (Nutter et al., 2013; Nutter and Cooper, 2014). These molecular adaptations persisted 8 weeks after neurotoxicant exposure had ceased.

The K_v7 family of ion channel proteins belongs to the large family of K_v (voltage sensitive K^+) proteins that contribute to neural excitability and conduction in multiple ways (Du and Gamper, 2013; Tsantoulas and McMahon, 2014). The adaptations exhibited by K_v7 channels, following GW chemical exposure, could represent a general adaptation affecting K_v channels or a more specific adaptation of the K_v7 family through their linkages to muscarinic receptors whose expression may have been perturbed by anticholinesterases (Abou-Donia et al., 2003, 2004; Passmore et al., 2012). Because a variety of K_v channels have been implicated in the development of chronic pain, determining the scope of this defect could aid in the interpretations of determinant factors contributing to the development and treatment of GWI pain syndromes (Du and Gamper, 2013; Tsantoulas and McMahon, 2014). In the experiments presented below, we used an intensified anticholinesterase exposure protocol to induce a delayed pain behavior syndrome, and examined whether K_v7 and K_{DR} currents in deep tissue nociceptors were modified in a manner consistent with delayed chronic pain.

2. Methods

2.1. Behavioral studies

2.1.1. Subjects

Young adult male rats weighing 229.4 ± 2.6 g (~6 weeks old) were used in the pesticide exposure studies (Sprague-Dawley; Harlan; $n = 61$). All animals were housed in American Association for Accreditation of Laboratory Animal Care approved quarters, and all procedures were reviewed and approved by the local Institutional Animal Care and Use Committee and ACURO (Animal Care and Use Review Office of the Army Medical Research and Materiel Command). No animals perished or exhibited signs of acute pesticide toxicity during the execution of these studies.

2.1.2. Chronic exposure protocol

Over a period of 30 or 60 days, rats ($n = 30$) were exposed to permethrin (2.6 mg/kg; mixture of 26.4% cis and 71.7% trans; Sigma Aldrich), chlorpyrifos (120 mg/kg; Sigma Aldrich), and pyridostigmine bromide (PB; 13 mg/kg). Permethrin, in ETOH, was applied every day to a shaved area of the back between the forelimbs. Chlorpyifos was administered by a subcutaneous injection (corn oil) once every 7 days. The dose of chlorpyrifos was intended to represent a net exposure to the potentially large and varied anticholinesterases that soldiers were exposed to in the Gulf theater (Binns et al., 2008). Chlorpyrifos was administered in a corn oil formulation that released agent over a couple of days (Smith et al., 2009). PB was administered daily by oral gavage (tap water) based upon a standard military dose that was adjusted to account for faster pharmacokinetics in rodents (Birtley et al., 1966; Husain et al., 1968; Aquilonius et al., 1980; Breyer-Pfaff et al., 1985). Rats were weighed once per week throughout the studies and doses were adjusted accordingly. Control rats received only vehicle exposures over the identical time course ($n = 31$).

Rats were sacrificed for electrophysiological studies 8 and 12 weeks after chemical exposures had ended. All rats underwent behavioral testing before, during and after chemical exposures (see below). There was little indication that chemical exposures affected body weight. The average body weights of vehicle and chemically exposed rats did not differ at the 8-week post-exposure period (vehicle: 443.4 ± 9.1 and exposed: 430.8 ± 8.6 g; $p < .35$).

2.1.3. Assessment of pain behaviors

On arrival, rats were acclimated to the behavioral procedures for 2 weeks before dosing began. Testing continued weekly throughout the entire dosing and post-dosing periods. Pressure pain withdrawal thresholds were measured using a computer monitored, hand held test device (PAM; Ugo Basile). Pressure was applied *via* a 5 mm diameter ball force transducer to the semitendinosus and biceps femoris muscles (right hind limb). During force application, the applied pressure was monitored and displayed to the experimenter on a video screen. Video feedback enabled the rate of force application to be regulated according to a visual standard. When the rat withdrew its limb, the force at withdrawal was automatically registered and stored. To complement pressure pain testing, activity levels (movement distance, movement rate, and rest times) were recorded automatically by infrared sensors in an activity box (15 min test period; Fusion System, AccuScan Instruments Inc.). Behavior tests were conducted on both chemically exposed (permethrin, chlorpyrifos, PB) and vehicle treated (ETOH, corn oil, water) animals over an identical time course. Rats were tested once per week on the behavioral tasks. Tests were conducted in 'blinded' conditions.

2.2. Electrophysiological studies

2.2.1. Preparation of cells

Dorsal root ganglion neurons (DRG) were harvested from chemically and vehicle exposed rats 8 and 12 weeks after termination of chemical exposures. Rats were anesthetized (isoflurane) and rapidly euthanized by decapitation (Harvard Instruments). The spinal column was removed, bisected and the DRG were dissected free from T11 to S1. Ganglia were trimmed, cut into strips and digested in Tyrode's solution containing collagenase A (2 mg/ml) and dispase II (5 mg/ml; Roche Chemical). A 15 ml centrifuge tube containing the dissected ganglia was placed in a heated, shaking water bath for 90 min at 35 °C (EDVOTEK Digital Shaking Water Bath). Gentle trituration was then used to break up visible strips of ganglia. The dispersed neurons were then digested for an additional 45 min, and then spun at 1000 RPM (30 s). The supernatant was discarded. The remaining pellet was dispersed into 2 ml of Tyrode's, triturated and plated on 8–10, 35 mm, polylysine coated, Petri dishes (Fluorodish). Plated neurons were bathed continuously in a Tyrode's solution, containing (in mM) 140 NaCl, 4 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH. All electrophysiological studies were conducted at room temperature (21 °C) within 10 h of plating. Only one cell was used per Petri dish. Electrodes were formed from boroscilate glass stock that was pulled to a suitable tip resistance (2–4 MΩ) by a Sutter P1000 (Sutter Instruments, Novato, CA). The pipette solution contained (in mM): 120 KCl,