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Exposure to Gulf War Illness chemicals induces functional muscarinic receptor maladaptations in muscle nociceptors

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ABSTRACT

Chronic pain is a component of the multisymptom disease known as Gulf War Illness (GWI). There is evidence that pain symptoms could have been a consequence of prolonged and/or excessive exposure to anticholinesterases and other GW chemicals. We previously reported that rats exposed, for 8 weeks, to a mixture of anticholinesterases (pyridostigmine bromide, chlorpyrifos) and a Na_v (voltage activated Na^+ channel) deactivation-inhibiting pyrethroid, permethrin, exhibited a behavior pattern that was consistent with a delayed myalgia. This myalgia-like behavior was accompanied by persistent changes to K_v (voltage activated K^+) channel physiology in muscle nociceptors (K_v7, K_{DR}). In the present study, we examined how exposure to the above agents altered the reactivity of K_v channels to a muscarinic receptor (mAChR) agonist (oxotremorine-M). Comparisons between muscle nociceptors harvested from vehicle and GW chemical-exposed rats revealed that mAChR suppression of K_v7 activity was enhanced in exposed rats. Yet in these same muscle nociceptors, a Stromatoxin-insensitive component of the K_{DR} (voltage activated delayed rectifier K^+ channel) exhibited decreased sensitivity to activation of mAChR. We have previously shown that a unique mAChR-induced depolarization and burst discharge (MDBD) was exaggerated in muscle nociceptors of rats exposed to GW chemicals. We now provide evidence that both muscle and vascular nociceptors of naïve rats exhibit MDBD. Examination of the molecular basis of the MDBD in naïve animals revealed that while the mAChR depolarization was independent of K_v7, the action potential burst was modulated by K_v7 status. mAChR depolarizations were shown to be dependent, in part, on TRPA1. We argue that dysfunction of the MDBD could be a functional convergence point for maladapted ion channels and receptors consequent to exposure to GW chemicals.

1. Introduction

Following the 1991 Persian Gulf War, thousands of returning soldiers developed a syndrome comprised of a varying mixture of cognitive, motor, sensory and autonomic dysfunctions that came to be known as Gulf War Illness (GWI; Binns et al., 2008; Haley et al., 2013; White et al., 2016). A large portion of those suffering from GWI complained of chronic pain symptoms that were described as a mixture of headache, back pain, muscle, joint, and abdominal pains (Haley and Kurt, 1997; Blanchard et al., 2006; Stimpson et al., 2006; Thomas et al., 2006). While symptoms typically arose after warfighters had returned from their deployments, a significant portion of veterans (25%) reported symptoms of GWI while still in theater (Kroenke et al., 1998). In succeeding years, the severity of GWI symptoms tended to remain constant or worsen (Hotopf et al., 2003).

Diverse risk factors, including exposure to Sarin gas, depleted uranium, oil fires, vaccination adjuvants, organophosphates and combat stress have been proposed as factors contributing to the development of GWI. However, no single factor has been able to account for the wide ranging symptoms of this complex multi-symptom disease. The Research Advisory Committee on Gulf War Illness concluded that pesticides could have contributed to the development of the symptoms of GWI (Binns et al., 2008; RAC, 2014). While deployed to the Persian Gulf, soldiers were potentially exposed to 67 insecticides and repellants that contained 37 distinct ingredients (DoD Environmental Exposure Report: Pesticides, 2003; Binns et al., 2008). Organophosphate, organochlorine, dialkylamide, carbamate and pyrethroid pesticides and repellants were used liberally in the Gulf theater.

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Coincident with exposure to these agents, warfighters were self-administering a nerve gas prophylactic, pyridostigmine bromide (PB), which shares the anticholinesterase properties of many insecticidal chemicals. Pesticides were to be used at specific concentrations and with specific application methods and procedures. These application procedures were not always carefully followed and warfighters often supplemented designated agents with others they acquired on their own (US, DoD Environmental Exposure Report: Pesticides, 2003). While the levels of exposure to any one of these pesticides may not have posed a significant detriment to the health of the troops, synergisms arising from multiple chemical exposures could have converged on one or more molecular targets to produce long lasting physiological maladaptations and/or physical damage to nervous system components.

Utilizing a variety of exposure protocols under laboratory conditions, it has been demonstrated that combinations of permethrin, chlorpyrifos, pyridostigmine bromide and other GW chemicals, produce a variety of motor and cognitive signs (Servatius et al., 1998; Servatius et al., 2000; Abou-Donia et al., 2001; Abdel-Rahman et al., 2004a; Abou-Donia et al., 2004; Parihar et al., 2013), induce inflammatory agents (Li et al., 2001; Singh and Jiang, 2003; Terry, 2012), cause microvascular injury (Ojo et al., 2014), degrade the blood brain barrier (Grauer et al., 2001; Abdel-Rahman et al., 2002; Abdel-Rahman et al., 2004b), suppress enzyme activity (Abdel-Rahman et al., 2002; Casida and Quistad, 2005), and damage DNA (Falcioni et al., 2010). However, a laboratory model suitable for the study of GWI related chronic pain has been elusive (Scremin et al., 2003; see also Lotti and Moretto, 2005).

Several years ago we began a series of studies that were designed to reproduce the myalgia and arthralgia of GWI in a rat model. Certain GW chemicals have properties that could directly (pyrethroids) or indirectly (anticholinesterases) interact with important membrane ion channel and receptor proteins expressed in peripheral nociceptors (e.g., voltage activated Na^+ channel 1.8 ($Na_v1.8$): Narahashi et al., 1998; Soderlund et al., 2002; Bradberry et al., 2005; Ray and Fry, 2006; Jiang et al., 2013; Muscarinic receptors: Abou-Donia et al., 2003, 2004; voltage activated K^+ channel 7 (K_v7): Marrion, 1997; Robbins, 2001; Brown and Passmore, 2009). Following a 60 day exposure to the anticholinesterases pyridostigmine bromide and chlorpyrifos and the pyrethroid insecticide permethrin, we discovered that the physiology of vascular and/or muscle nociceptor ion channel proteins $Na_v1.9$ and K_v7 were altered 8–12 weeks after exposures had ceased (Nutter et al., 2013; Nutter and Cooper, 2014). In contrast, the physiology of $Na_v1.8$ was unaffected in these nociceptor pools (Nutter et al., 2013). Despite a relatively consistent pattern of perturbed ion channel physiology, we could not demonstrate behavioral changes consistent with the development of a chronic pain syndrome.

Recently, we reported that a revised exposure protocol, modified so as to increase the frequency of anticholinesterase exposure, did have a substantial effect on post-exposure rat behavior patterns. Disturbances in movement and rest patterns that evolved over a 12 week observation period suggested the development of a delayed myalgia/arthralgia. Molecular studies tended to support this interpretation, as K_v7 and other K_{DR} (voltage activated delayed rectifier K^+ channel) currents were greatly diminished in muscle nociceptors during the manifestation of pain-like signs. Moreover, action potential bursts induced by muscarinic agonists were significantly increased during periods of heightened pain-like behaviors (Nutter et al., 2015). The emergence of pain signs following an increased frequency of exposure to chlorpyrifos and PB suggested that anticholinesterases played a critical role of in the development of some GWI related pain. The changes we observed in the physiology of K_v7 proteins could reflect changes in expression of these channels or alteration in the pathways that regulate their activity.

Exposure to chlorpyrifos or PB can increase expression of muscarinic receptors in neocortex (Abou-Donia et al., 2003, 2004). Potentially, the expression of muscarinic receptors (mAChR) could have been altered in DRG (dorsal root ganglion) following exposure to chlorpyrifos and PB. In the experiments described below, we examined whether a 60 day exposure to permethrin, chlorpyrifos and PB altered nociceptor K^+ channel reactivity to a muscarinic agonist, and whether the pattern of alteration was consistent with the development of a GWI pain syndrome.

2. Methods

2.1. Behavioral studies

2.1.1. Exposure protocol

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. Juvenile male rats, initially weighing between 90 and 110 g, were used in all studies (chronic studies: n = 35; acute studies: n = 17; Sprague-Dawley; Harlan/Envigo). In chronic experiments, 15 rats 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), for 60 days. 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. PB was given daily by oral gavage (tap water). The latter represented a standard military dose of PB (assuming a 70 kg body weight). Twenty additional rats received only vehicle exposures using an identical administration schedule. Rats were sacrificed for electrophysiological studies 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 (442.4 ± 5.0 g; n = 20, Vehicle; and 430.8 ± 8.6 g; n = 15, Exposed; $p < 0.28$). Rats were weighed once per week and doses were adjusted according to body weight.

2.1.2. Assessment of pain behaviors

On arrival, rats were acclimated to the behavioral procedures for 2 weeks before dosing began. Testing continued 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 (left 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). Behavior tests were conducted on both chemical-exposed (permethrin, chlorpyrifos, PB) and vehicle treated (ETOH, corn oil, water) animals over an identical time course. From baseline testing (Fig. 1) until they were sacrificed, the rats were tested once per week on the 4 behavioral tasks. In order to avoid potential experimenter test bias, all the manual PAM tests were conducted under 'blinded' conditions (with the exception of