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Molecular and cellular influences of permethrin on mammalian nociceptors at physiological temperatures

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ABSTRACT

The influence of pyrethroid insecticides is thought to be abrogated at mammalian physiological temperatures. Yet there are many reports of transient pain and paresthesia following accidental exposures. Using whole cell patch clamp techniques, we examined the interaction of the pyrethroid insecticide permethrin on skin, muscle and putative vascular nociceptors of the rat DRG (dorsal root ganglion). Following permethrin (10 μM) application, action potential (AP) duration was increased in all nociceptor populations, but only muscle nociceptors developed spontaneous activity or increased excitability (tests at 21 °C). TTX (tetrodotoxin) did not prevent the development of spontaneous activity or reduce excitability. We examined the influence of permethrin on TTX resistant channel proteins that control excitability and spontaneous activity ($Na_v1.8$, voltage-gated sodium channel 1.8; K_v7, voltage gated potassium channel 7). In all nociceptor populations, permethrin increased the tau of deactivation (taudeact), in a voltage dependent manner, and hyperpolarized the $V_{1/2}$ for activation over 10 mV. There were no permethrin dependent influences on K_v7, or on the voltage dependence of inactivation of $Na_v1.8$. The influence of permethrin on AP duration, after hyperpolarization, spontaneous activity, half-activation potential ($V_{1/2}$) and taudeact were reduced, but not fully reversed, when tests were conducted at 35 °C. In conclusion, permethrin greatly modifies the voltage dependent activation and deactivation of $Na_v1.8$ expressed in skin, muscle and vascular nociceptors. These influences remain significant at 35 °C. One population of muscle nociceptors exhibited a unique vulnerability to the acute administration of permethrin manifested as increased excitability and spontaneous activity.

1. Introduction

In humans, burning and stinging sensations have been reported following either ingestion (Gotoh et al., 1998) or acute topical contact with the type I pyrethroid insecticide, permethrin (Kolmodin-Hedman et al., 1982; Tucker and Flannigan, 1983; Flannigan and Tucker, 1985) or other pyrethroid formulations (Knox et al., 1984; Wilks, 2000; see also Wolansky and Harrill, 2008). Specific pyrethroids (permethrin and allethrin) have also been linked to the development of certain chronic pain states (Binns et al., 2008). The influence of type I and type II pyrethroids (allethrin, tetramethrin and deltamethrin) on TTXs and TTXr Na_v proteins of the DRG suggest a relatively direct pathway to nociceptor activation and pain (Ginsburg and Narahashi, 1993; Tatebayashi and Narahashi, 1994; Song et al., 1996; Song and Narahashi, 1996a,b; Tabarean and Narahashi, 1998, 2001). However, the apparent influence of pyrethroids on mammalian nociceptors has not been directly demonstrated, and stands in contrast with the supposed mitigating influences of body temperature and other factors that make it safe for use near humans.

It is well established that both type 1 and type 2 pyrethroid insecticides alter the physiology of voltage-gated sodium channels (VGSC, or Na_v). While there have been few studies on the influence of the type 1 pyrethroid, permethrin, on neural channel protein function, the influence of structurally similar pyrethroid neurotoxicants have been studied extensively. In insects, pyrethroids induce or obstruct neural activity via modulation of activation, steady-state inactivation and deactivation of Na_v proteins (Narahashi et al., 1998; Narahashi, 2000; Ray and Fry, 2006; Soderlund, 2010). Early investigations of interactions with sensory neurons of the DRG (dorsal root ganglion), confirmed powerful influences between several pyrethroids (allethrin, tetramethrin, deltamethrin) and structurally similar mammalian Na_v (Ginsburg and Narahashi, 1993; Tatebayashi and Narahashi, 1994; Song et al., 1996; Tabarean and Narahashi, 1998, 2001). Altered activation, steady-state inactivation and deactivation of Na_v proteins have

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been reported across the diverse family of mammalian TTXs (tetrodotoxin sensitive) and TTXr (tetrodotoxin resistant) Na_v (e.g., $Na_v1.2$, $Na_v1.3$, $Na_v1.6$, $Na_v1.8$), but the specifics of modulation by type I and type II pyrethroids differed both qualitatively and quantitatively (Song et al., 1996; Smith and Soderlund, 1998; Motomura and Narahashi, 2001; Dekker et al., 2005; Choi and Soderlund, 2006; Meacham et al., 2008; Breckenridge et al., 2009; Tan and Soderlund, 2009, 2010).

Mammals are highly resistant to the toxic influences of pyrethroids. The absence of lethal effects on mammals is believed to be due, in part, to a powerful temperature dependence of pyrethroid actions on Na_v that substantially reduces their influence at body temperature. Other factors such as body size, catabolic rates and innate sensitivity also contribute to large safety factors associated with pyrethroids (Soderlund et al., 2002; Bradberry et al., 2005; Ray and Fry, 2006). While some temperature-dependent effects of pyrethroids have been documented, many aspects of these interactions have not been examined. Using a preparation of central nervous system neurons that expressed the fast inactivating TTXs Na_v channel proteins, Song and Narahashi demonstrated that the slowing of TTXs Na_v deactivation by tetramethrin was not completely reversed at 35 °C (Song and Narahashi, 1996b). Other important pyrethroid influences (voltage dependent activation and inactivation) were not examined at elevated temperatures. Moreover, the action potentials of mammalian nociceptors are formed by TTXr $Na_v1.8$ (Djouhri et al., 2003a,b; Jiang and Cooper, 2011). The thermal dependence of pyrethroids, on TTXr channels, has never been examined at physiological temperatures. At 28 °C, allethrin retains the capacity to alter TTXr deactivation, but also exhibits other temperature independent modifications of $Na_v1.8$ physiology (Ginsburg and Narahashi, 1999).

Accordingly, we initiated a series of studies to examine how permethrin differentially interacted with specific skin, muscle and putative vascular nociceptors that express the pyrethroid sensitive TTXr protein $Na_v1.8$. Experiments were conducted at both room and physiological temperatures. We observed that permethrin had predictable influences on $Na_v1.8$ across superficial and deep nociceptor families, but that the cellular consequences of this interaction differed. Moreover, substantial influences of permethrin on voltage dependent activation, deactivation and action potential characteristics were still evident at 35 °C.

2. Methods

2.1. Preparation of cells

Young adult male Sprague–Dawley rats (n = 95; 90–110 g) were anesthetized (isoflurane) and euthanized by decapitation. The dorsal root ganglia were excised, digested in a solution containing type 1 collagenase and dispase II. The procedure has been described in detail previously (Petruska et al., 2002). Isolated neurons were plated on 8–10, 35 mm Petri dishes. Neurons were bathed continuously in 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. Electrodes were formed from borosilicate 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, 5 Na_2–ATP, 0.4 Na_2–GTP, 5 EGTA, 2.25 $CaCl_2$, 5 $MgCl_2$, 20 HEPES, adjusted to pH 7.4 with KOH. The osmolarity was approximately 290 mOsm. Recordings were completed within 2–10 h after plating. Only one cell was used per Petri dish. Studies were conducted at room temperature unless otherwise noted. 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.

2.2. Recording and characterization of skin, muscle and vascular nociceptors

Whole cell recordings were made with an Axopatch 200B or Multiclamp 700 (Molecular Devices, Sunnyvale, CA). Stimuli were controlled and records were captured with pClamp software and Digidata 1322A (Molecular Devices). Series resistance (R_s) was compensated 65–70% with Axopatch compensation circuitry. Whole cell resistance and capacitance were determined by the Clampex software utility. Recorded currents were sampled at 10–20 kHz and filtered at 2 kHz (Bessel filter).

Classification of nociceptors into 'types' was performed by presentation of a series of depolarizing or hyperpolarizing characterization protocols originally developed by Scroggs and extended by Cooper (Cardenas et al., 1995; Petruska et al., 2000, 2002). All reported data were derived from recordings made on type 2 (C nociceptor), type 5 (C or type IV nociceptor), and type 8 (Aδ or type IV nociceptor). Type 2 and type 5 were identified as skin and muscle nociceptors, respectively, in tracing studies (Jiang et al., 2006; Rau et al., 2007, 2012). Type 8 nociceptors were traced from all tissue sites (skin, muscle, colon, mucosa; Rau et al., 2007, 2012) and are presumed to be vascular in origin.

2.3. Permethrin application

Agents were applied by close superfusion (sewer pipe). An all glass superfusion system was devised to avoid complications due to the known binding of highly lipophilic pyrethroids to plastics (Tatebayashi and Narahashi, 1994; Shafer and Hughes, 2010). Ten milliliter glass syringe reservoirs were coupled to a small Plexiglas manifold by a flexible glass tubing system terminating in a 'sewer pipe' that was positioned within 2 mm of the targeted cells. The glass tubing consisted of 3 rigid sections of 150 mm glass (.86 mm inner diameter, 1.5 mm outer diameter; Sutter Instruments). Each rigid piece was linked by a 3 mm section of silastic tubing. Only about 1 mm of each silastic linker was in contact with the permethrin containing solution. The Plexiglas manifold was not exposed to permethrin until the actual application occurred. Following each application of permethrin, the manifold was rinsed for at least 2 min with 100% ETOH. At the termination of each experiment, the plastic toggle that was exposed to permethrin was discarded and flexible glass tubing was rinsed with 10 ml of 100% ETOH. To equalize flow rates, the glass application system was used to apply all agents. Stock solutions of permethrin were prepared and maintained in glass bottles (racemic mixture of 26.4% cis and 71.7% trans in ETOH vehicle; Sigma Aldrich). In studies in which heated solutions were used, a feedback controlled bath controller (Model TC_{bip}) and Model HPRE applicator (Cell MicroControls, Norfolk, VA) was used to pre-heat the superfused solution to the desired temperature.

2.4. Action potential duration, excitability and spontaneous activity experiments

In current clamp mode, an action potential was evoked by a brief, high amplitude, current injection (1 ms, 3 nA; 3 replications). Action potential duration was measured as the width of the action potential at its base (Petruska et al., 2002). Excitability was assessed by a series of stepped current injections (0.1–1 nA; 250 ms; 10 consecutive steps). Excitability was quantified as the total number of action potentials evoked in the 10 stepped current injections. The resting membrane potential was adjusted to