NeuroToxicology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

NeuroToxicology

Full length article

Developmental pyrethroid exposure causes long-term decreases of neuronal sodium channel expression

Jason P. Magby[a], Jason R. Richardson[a,b,*]

[a] Environmental and Occupational Health Sciences Institute and Department of Environmental and Occupational Medicine, Robert Wood Johnson Medical School, Rutgers University, Piscataway, NJ 08854, USA
[b] Northeast Ohio Medical University Department of Pharmaceutical Sciences, Rootstown, OH 44272, USA

ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form 24 March 2016
Accepted 1 April 2016
Available online xxx

Keywords:
Pyrethroid
Deltamethrin
Sodium channel
BDNF
Neurodevelopmental

ABSTRACT

Pyrethroid insecticide use has increased over recent years because of their low to moderate acute toxicity in mammals. However, there is increasing concern over the potential detrimental effects of pyrethroids on developing animals. Most recently, we have shown that developmental exposure to deltamethrin results in long-term neurobehavioral effects. Pyrethroids exert their toxicity by acting on the voltage-gated sodium channel (Na_v), delaying channel inactivation and causing hyperexcitability in the nervous system. Previous *in vitro* studies found that exposure to agents that increase Na^+ influx, including deltamethrin decreased Na_v mRNA expression. However, it is unknown whether this occurs *in vivo*. To determine whether developmental pyrethroid exposure decreases Na_v mRNA expression, pregnant mice were exposed to the pyrethroid deltamethrin (0 or 3 mg/kg) every three days throughout gestation and lactation. Na_v mRNA expression was measured in the striatum and cortex of the offspring at 10–11 months of age, a time at which behavioral abnormalities were still observed. Developmental exposure to deltamethrin decreased expression of Na_v mRNA in a region- and isoform-specific fashion by 24–50%. Deltamethrin exposure also resulted in the persistent down-regulation of brain-derived neurotrophic factor (Bdnf) in the striatum by 66% but not in the cortex, suggesting a plausible mechanism for some of the associated behavioral effects observed previously. Taken together these data suggest that developmental deltamethrin exposure results in persistent deficits in Na_v and BDNF mRNA expression that may contribute to long-term behavioral deficits.

1. Introduction

Pyrethroid insecticides are potent neurotoxic insecticides that account for over 25% of the total annual pesticide use in the world (Casida and Quistad, 1998; Horton et al., 2011; Morgan, 2012). The primary mechanism by which pyrethroids exert their neurotoxic effects in insects involves delaying the inactivation of voltage-gated sodium channels (Na_v) (Narahashi, 1971). This delayed inactivation increases sodium influx, action potential generation, neuronal excitability and ultimately leads to conduction block, particularly with Type II pyrethroids, convulsions and death, if the dose is high enough (Narahashi, 1996).

Na_v are integral membrane proteins principally composed of a large (~260 kDa) α subunit that forms a central ion-conducting pore and one or more smaller auxiliary β subunits that are important modifiers of channel gating kinetics, cell-surface expression, and cell-cell interactions (Isom, 2001). The mammalian genome contains 10 sodium channel α subunit isoforms and four β subunits isoforms, yielding multiple subunit combinations, some of which are differentially sensitive to the effects of pyrethroids (Meacham et al., 2008; Soderlund, 2012). In an oocyte expression system, deltamethrin has the most pronounced effect on sodium currents through $Na_v1.3/\beta3$ (Meacham et al., 2008) and $Na_v1.6/\beta1+\beta2$ channels (Tan and Soderlund, 2010). This may be particularly important to the potential developmental neurotoxicity of deltamethrin, as $Na_v1.3/\beta3$ channel complexes are highly expressed in the developing rodent brain (Albrieux et al., 2004) and the $Na_v1.6$ isoform is abundantly expressed in the nodes of Ranvier, dendrites, and synapses (Caldwell et al., 2000).

Although there is a general lack of information in the literature describing the developmental neurotoxicity of pyrethroids (Shafer et al., 2005), developing animals are more susceptible to the acute toxic effect of type II pyrethroids (Sheets et al., 1994). This greater susceptibility has been ascribed to lower metabolic detoxication enzymes compared to adults (Cantalamessa, 1993; Sheets et al., 1994). However, the greater abundance of the highly sensitive

* Corresponding author at: 4209 St. Rt. 44, RGE-136, Rootstown, OH 44272, USA.
E-mail address: jrichardson@neomed.edu (J.R. Richardson).

http://dx.doi.org/10.1016/j.neuro.2016.04.002
0161-813X/

$Na_v1.3/\beta3$ channels during neuronal development may also contribute to the increased susceptibility of the developing central nervous system (Meacham et al., 2008). Determining mechanism (s) of the potential developmental neurotoxicity of pyrethroids is particularly important because there is documented exposure of pregnant women and children to pyrethroids (Whyatt et al., 2002; Berkowitz et al., 2003; Heudorf et al., 2004; Bouwman et al., 2006; Barr et al., 2010), and the number of pyrethroid poisonings in children reported to poison control centers has increased recently (Power and Sudakin, 2007). More recently, we reported higher levels of pyrethroid metabolites in the urine of children increased likelihood of ADHD diagnosis (Wagner-Schuman et al., 2015) and that low-level deltamethrin exposure of mice during gestation resulted in hyperactivity and impulsive-like behavior in their male offspring (Richardson et al., 2015).

Previous *in vitro* studies found that exposure to compounds that delay Na_v inactivation, such as veratridine and scorpion toxin, decreased Na_v protein levels (Dargent and Couraud, 1990) and mRNA expression (Lara et al., 1996; Magby and Richardson, 2015), possibly as a compensatory mechanism to reduce hyperexcitability in the cells. The mechanism of this down-regulation is not well established, but may include calpain activation. Additionally, *in vitro* studies reported that the down-regulation of sodium channels occurs only in brain slices from immature animals and not in slices obtained from adult animals (Dargent et al., 1994). These findings suggest a unique susceptibility of the developing nervous system to agents that increase Na_v influx that is likely independent of differences in detoxication capacity. However, it is not known whether *in vivo* exposure to agents that delay Na_v inactivation, such as pyrethroids, causes similar effects.

In this study, we report that developmental exposure to the pyrethroid deltamethrin results in long-term down-regulation of Na_v mRNA expression *in vivo*. This down-regulation is correlated with decreased brain-derived neurotrophic factor (BDNF) an important growth factor that is regulated by neuronal activity (Lu, 2003; Imamura et al., 2006; Sharma et al., 2008; Hara et al., 2009; Ihara et al., 2012). Thus, developmental exposure to deltamethrin appears to result in long-term changes in Na_v expression, which could contribute to the persistent alterations of neuronal function and long-term behavioral deficits observed in our previous study.

2. Materials and methods

2.1. Chemicals

Deltamethrin was purchased from ChemService (99.5% purity and lot 418-66B; West Chester, PA). All other reagents were purchased from Sigma-Aldrich unless otherwise noted.

2.2. In vivo *exposure*

Male and female C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME) at 6–8 weeks of age. Mice were maintained on a 12:12 light/dark cycle with food and water available ad libitum. All procedures were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Rutgers-Robert Wood Johnson Medical School.

Breeding and deltamethrin treatments were performed as described previously (Richardson et al., 2015). Briefly, pregnant female mice were individually housed and fed 0 or 3 mg/kg deltamethrin dissolved in corn oil and administered in a small amount of peanut butter every 3 days throughout gestation and lactation, starting at gestation day 6 and ending at weaning on PND25. This dosing paradigm was used to reduce handling stress on the pregnant female (Caudle et al., 2005) and the dose administered every 3 days to allow for significant clearance of the compound (Ruzo et al., 1979). This dose of deltamethrin is lower than the developmental NOAEL (12 mg/kg;(Kavlock et al., 1979)) and was determined based on our previous work that demonstrated no overt toxicity to the dam or offspring (Armstrong et al., 2013). Male mice were sacrificed by decapitation at approximately 10–11 months of age (n = 5–7 with each animal representing an individual litter). These mice were littermates of those previously reported to display hyperactivity and impulsive-like behavior at this time point (Richardson et al., 2015). Males were chosen for these experiments to eliminate the possibility of estrogen-related cyclicity, as estrogen levels affect Na_v expression (Hu et al., 2012; Wang et al., 2013), and because of the male predominant behavioral deficits observed following developmental deltamethrin exposure (Richardson et al., 2015). Brains were removed, and the frontal cortex and striatum dissected on ice, and frozen in liquid nitrogen. Samples were stored at −80 °C until RNA isolation.

2.3. Quantitative real-time polymerase chain reaction (qPCR)

qPCR was performed as described previously (Richardson et al., 2008; Fortin et al., 2013). Briefly, total RNA was isolated using Qiagen RNeasy mini kits and RNA (0.5 μg) was reverse-transcribed using Superscript II (Invitrogen, Carlsbad, CA). QPCR reactions were performed in duplicate using an ABI 7900HT and SYBR Green (Applied Biosystems, Carlsbad, CA) detection. β-actin was used as the normalizing gene and data were calculated using the $2^{\Delta\Delta Ct}$ method as described previously (Richardson et al., 2008). Primers were designed using the Primer Blast program (NCBI) and are listed in Supplemental Table 1.

2.4. Statistical analysis

All statistical analyses were performed on raw data. Data were analyzed using Student's *t*-test to determine effects on individual isoforms within a single brain region.

3. Results

Developmental deltamethrin exposure did not result in alteration of maternal weight gain during pregnancy nor body weight of the offspring at sacrifice (Supplemental Fig. 1). Likewise, no overt signs of pyrethroid toxicity were observed in the dam or offspring.

3.1. Regional differences in Na_v gene expression in the cortex and striatum

As an initial step in characterizing the effects of developmental deltamethrin exposure on Na_v expression in 10–11 month old offspring, we first assessed whether there were regional differences in Na_v isoform expression. The relative abundance of Na_v isoform expression was $Na_v1.2 > Na_v1.6 > Na_v1.1 > Na_v1.3$, regardless of brain region examined. Comparing the two regions, in the cortex, $Na_v1.2$ (11%) and $Na_v1.6$ (21%) were more highly expressed, while $Na_v1.1$ (40%) and $Na_v1.3$ (54%) were more highly expressed in the striatum (Fig. 1) when normalized to cortex. The relative abundance of β subunits in the cortex was $Nav\beta2 > Nav\beta1 > Nav\beta3 > Nav\beta4$ whereas the striatum $Nav\ \beta2 > Nav\ \beta1 > Nav\ \beta4 > Nav\beta3$. Comparing the two regions the $Nav\beta2$ (50%) and $Nav\beta4$ (12x fold) were more highly expressed in the striatum than in the cortex, when normalized to cortex (Fig. 1).

Please cite this article in press as: Magby, J.P., Richardson, J.R., Developmental pyrethroid exposure causes long-term decreases of neuronal sodium channel expression. Neurotoxicology (2016), http://dx.doi.org/10.1016/j.neuro.2016.04.002