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Full Length Article

Age-dependent behaviors, seizure severity and neuronal damage in response to nerve agents or the organophosphate DFP in immature and adult rats

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ABSTRACT

Exposure to nerve agents (NAs) and other organophosphates (OPs) can initiate seizures that rapidly progress to status epilepticus (SE). While the electrographic and neuropathological sequelae of SE evoked by NAs and OPs have been characterized in adult rodents, they have not been adequately investigated in immature animals. In this study postnatal day (PND) 14, 21 and 28 rat pups, along with PND70 animals as adult controls, were exposed to NAs (sarin, VX) or another OP (diisopropylfluorophosphate, DFP). We then evaluated behavioral and electrographic (EEG) correlates of seizure activity, and performed neuropathology using Fluoro-Jade B. Although all immature rats exhibited behaviors that are often characterized as seizures, the incidence, duration, and severity of the electrographic seizure activity were age-dependent. No (sarin and VX) or brief (DFP) EEG seizure activity was evoked in PND14 rats, while SE progressively increased in severity as a function of age in PND21, 28 and 70 animals. Fluoro-Jade B staining was observed in multiple brain regions of animals that exhibited prolonged seizure activity. Neuronal injury in PND14 animals treated with DFP was lower than in older animals and absent in rats exposed to sarin or VX. In conclusion, we found that NAs and an OP provoked robust SE and neuronal injury similar to adults in PND21 and PND28, but not in PND14, rat pups. Convulsive behaviors were often present independent of EEG seizures and were unaccompanied by neuronal damage. These differential responses should be considered when investigating medical countermeasures for NA and OP exposure in pediatric populations.

1. Introduction

Exposure to nerve agents (NAs) and other organophosphates (OPs) can produce prolonged repetitive seizures and status epilepticus (SE), a medical condition that causes high morbidity and mortality. NAs and other OPs irreversibly bind to the active site of acetylcholinesterase (AChE) and thus inhibit the degradation of the neurotransmitter acetylcholine, which then results in excess cholinergic activation (Aas, 2003). Centrally, excessive activation at muscarinic receptors by acetylcholine can initiate extended seizures (Hamilton et al., 1997), and these extended seizures can rapidly progress to SE which in turn produces marked neuronal death and mortality in animals (Apland et al., 2010; Crawford et al., 2004; Li et al., 2011; McDonough and Shih, 1997; Tuovinen, 2004).

The human pediatric population is thought to be more susceptible than adults to seizure development in general (Schaffer and Sirven, 2013; Volpe, 2008), and very young children (<2 yr old) are more likely to experience SE than older patients (Shinnar et al., 1997). Children who were acutely poisoned with carbamates or OPs displayed characteristic peripheral and central symptoms of anticholinesterase intoxication, with convulsive seizures appearing in 8–30% of cases (Lifshitz et al., 1999; Verhulst et al., 2002; Zwiener and Ginsburg, 1988). Similarly, in animal models, results

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from studies in which the chemoconvulsants pilocarpine or kainic acid were administered to immature and adult rats suggested that seizure susceptibility and mortality are greater in younger animals than they are in adults (Albala et al., 1984; Cavalheiro et al., 1987; Priel et al., 1996; Tremblay et al., 1984; Yang et al., 1998). Conversely, seizure-associated neuronal injuries in response to these agents increase as the age of the animal increases (Cavalheiro et al., 1987; Haut et al., 2004; Scantlebury et al., 2007). These data suggest that there are age-specific effects of NAs and other OPs. However, while age-related responses to other chemoconvulsant agents have been investigated (Cavalheiro et al., 1987; Haut et al., 2004; Scantlebury et al., 2007), and there are also electrographic and neuropathological characterizations of acute NA and OP poisoning in adult rats (Crawford et al., 2004; Deshpande et al., 2010; McDonough et al., 1995, 1998; Todorovic et al., 2012), characterizing the effects of these agents in immature animals has only just begun (Fawcett et al., 2009; Miller et al., 2015; Shih et al., 1990; Wright et al., 2016). As there are evident differences in the seizures of patients based on their age, therapies administered to adults many not be optimal for children (Baker, 2007; Rotenberg and Newmark, 2003). Therefore, an animal model of OP and NA exposure is required to evaluate the therapeutic efficacy of potential treatments in young individuals.

The current paper describes and validates age-appropriate models to evaluate OP- and NA-induced seizures in immature rats. Immature rat pups at postnatal day (PND) 14, 21, and 28, as well as adult rat controls (PND70), were administered the OP DFP or the NAs sarin or VX and then were examined for behavioral convulsions, electroencephalographic (EEG) evidence of seizure activity, and neuropathology. The results demonstrate that there is a strong relationship between age and both seizure susceptibility and neuronal damage, and that behavioral convulsive activity is not always predictive of EEG recorded seizures in immature animals.

2. Materials and methods

2.1. Animals

Pregnant Sprague-Dawley rats (at 13–15 days of pregnancy) were received from Charles River (Raleigh, NC); pups were delivered in the animal facility approximately 1 week following arrival of the pregnant female. Litters were culled to 8 or 10 pups to maintain consistent weights. Animals were kept on a 12-hr light-dark cycle and had access to food ad libitum. DFP administration was performed at the University of Utah, and NA administration procedures were performed at the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD). All surgical and experimental procedures were performed under protocols approved by the respective Institutional Animal Care and Use Committees at the University of Utah and USAMRICD, and were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and the Animal Welfare Act of 1966 (P.L. 89–544), as amended.

2.2. Implantation of EEG electrodes

University of Utah: Pups at PND12, 19, and 26, and adult rats at PND68 were anesthetized with 2.5–3% isoflurane and placed into a stereotaxic frame. An incision was made along the midline of the scalp to expose the skull. Burr holes were made using a high-speed dental drill. Electrode wires and the reference wire of the telemetric implant (Epitel, Inc., Salt Lake City, UT) were placed in the burr holes so that the wires were touching the dura, and were secured using cyanoacrylate gel compound with accelerator (Zayachkivsky et al., 2013). The incision was sutured shut using dissolvable suture. Post-surgery, the animals received 0.5–3 ml lactated Ringer's solution and 0.1 ml marcaine (MWI Veterinary Supplies, Boise, ID) or 0.015 mg/kg (PND12) or 0.03 mg/kg (PND19, 26 and 68) buprenorphine (MWI Veterinary Supplies, Boise, ID). All surgical procedures were performed under sterile conditions. The pups were allowed to recover in a heated recovery chamber until conscious before being returned to their dam (PND 12) or to a new cage (PNDs 19, 26, and 68).

USAMRICD: In animals that were administered NA, EEG recordings were made with a tethered system using implanted three-channel electrodes (Plastics One, Roanoke, VA) for the PND14, 21, and 28 groups or three cortical stainless-steel screw electrodes secured to a connector plug implant for the PND70 group. Pups at PND12 (PND14 group), 19 (PND21 group), and 25 or 26 (PND28 group), and adult rats PND63 or 64 (PND70 group) were anesthetized with isoflurane (5% induction; 0.5–3.0% maintenance, with oxygen) and placed into a stereotaxic frame. An incision was made along the midline of the scalp to expose the skull. For the PND14, 21, and 28 groups, the electrode wires were scraped bare of insulation, coiled and positioned in a triangular fashion flush with the skull, and the headpiece was secured using glass ionomer cement (Instech Laboratories, Plymouth Meeting, PA) or methyl methacrylate fast curing acrylic resin (Lang Dental, Wheeling, IL). In addition, two miniature anchoring screws were implanted in the skull to secure the headpiece in PND21 and 28 animals. For the PND70 group animals, burr holes were drilled over each hemisphere midway between bregma and lambda and 3 mm lateral to the midline, and an additional hole was drilled over the cerebellum. Stainless steel screw electrodes were placed in the holes and connected to a miniature plug via wires previously soldered to the screws; the whole assembly was then secured using glass ionomer or methyl methacrylate. Incisions were closed with non-absorbable monofilament suture material. Immediately after surgery all animals received warmed Ringer's solution (0.5–2 ml, s.c.) and PND14 pups received 0.015 mg/kg buprenorphine (s.c.), while the PND21, PND28 and PND70 animals received 0.03 mg/kg buprenorphine (s.c.), for analgesia. Animals of all age groups were placed in a heated recovery chamber until normal ambulation returned, and then were returned to their dam (PND14 group) or moved to a new cage (PND21, 28, and 70 groups).

2.3. NA and OP exposures and subsequent EEG recordings

University of Utah: Rat pups that received DFP (Sigma-Aldrich, St. Louis, MO) were pretreated with 0.026 mg/kg pyridostigmine bromide (Sigma-Aldrich, St. Louis, MO) i.p. 30 min before DFP treatment to reduce the peripheral effects of the OP. DFP was administered s.c. in ice-cold PBS in doses as follows: PND14, 4.0–4.5 mg/kg; PND21, 4.5-6.0 mg/kg; PND28, 4.0-6.5 mg/kg; and PND70, 4.0-6.25 mg/kg. At least three different batches of DFP were used for these experiments. Their potency in the animals was varied, even when fresh. Furthermore, aliquots of DFP stored at −80 °C would lose potency over several months. This resulted in a range of DFP doses that were employed to produce the greatest probability of animals exhibiting electrographic seizures without causing excess mortality. An admixture of 0.1 mg/kg atropine sulfate (Sigma-Aldrich, St. Louis, MO) and 25 mg/kg 2-PAM (Sigma-Aldrich, St. Louis, MO) was given i.p. 1 min after DFP administration to reduce respiratory distress. Behavioral responses were continuously observed for a minimum of 1 h following DFP administration in all age groups. EEG data were acquired using the Epoch™ wireless EEG system (Epitel, Inc., Salt Lake City, UT), and recording occurred for >30 min before and 3 h following DFP treatment for PND14 rat pups, and 24 h following DFP treatment for the PND21, PND28 and PND70 animals. EEG signals were amplified in the