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[+]-Huperzine A treatment protects against *N*-methyl-D-aspartate-induced seizure/*status epilepticus* in rats

Brian R. Coleman[a], Ruthie H. Ratcliffe[a], Samuel A. Oguntayo[a], Xuerong Shi[a], Bhupendra P. Doctor[a], Richard K. Gordon[a], Madhusoodana P. Nambiar[a,b,*]

[a] *Department of Biochemical Pharmacology/Division of Biochemistry, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500, United States*
[b] *Department of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, United States*

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ABSTRACT

The toxicity of organophosphorous (OP) nerve agents is attributed to their irreversible inhibition of acetylcholinesterase (AChE), which leads to excessive accumulation of acetylcholine (ACh) and is followed by the release of excitatory amino acids (EAA). EAAs sustain seizure activity and induce neuropathology due to over-stimulation of *N*-methyl-D-aspartate (NMDA) receptors. Huperzine A (Hup A), a blood–brain barrier permeable selective reversible inhibitor of AChE, has been shown to reduce EAA-induced cell death by interfering with glutamate receptor-gated ion channels in primary neuronal cultures. Although [−]-Hup A, the natural isomer, inhibits AChE approximately 38-fold more potently than [+]-Hup A, both [−]- and [+]-Hup A block the NMDA channel similarly. Here, we evaluated the protective efficacy of [+]-Hup A for NMDA-induced seizure in a rat model. Rats implanted with radiotelemetry probes to record electroencephalography (EEG), electrocardiography (ECG), body temperature, and physical activity were administered various doses of [+]-Hup A (intramuscularly) and treated with 20 μg/kg NMDA (intracerebroventricular) 20–30 min later. For post-exposure, rats were treated with [+]-Hup A (3 mg/kg, intramuscularly) 1 min after NMDA (20 μg/kg). Our data showed that pre- and post-exposure, [+]-Hup A (3 mg/kg) protects animals against NMDA-induced seizures. Also, NMDA-administered animals showed increased survival following [+]-Hup A treatment. [+]-Hup A has no visible effect on EEG, heart-rate, body temperature, or physical activity, indicating a reduced risk of side effects, toxicity, or associated pathology. Our results suggest that [+]-Hup A protects against seizure and *status epilepticus* (SE) by blocking NMDA-induced excitotoxicity *in vivo*. We propose that [+]-Hup A, or a unique combination of [+]- and [−]-Hup A, may prove to be effective for pre- and post-exposure treatment of lethal doses of OP-induced neurotoxicity.

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Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; ATCh, acetylthiocholine iodide; BChE, butyrylcholinesterase; CWNA, chemical warfare nerve agent; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); EAA, excitatory amino acid; ECG, electrocardiography; EEG, electroencephalography; GD, soman; Hup A, Huperzine A; icv, intracerebroventricular; im, intramuscular; *iso*-OMPA, tetraisopropyl pyrophosphoramide; NMDA, *N*-methyl-D-aspartate; OP, organophosphate; PCP, phencyclidine; SE, *status epilepticus*.

* Corresponding author at: Department of Biochemical Pharmacology, Division of Biochemistry, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Silver Spring, MD 20910-7500, United States. Tel.: +1 301 319 9679; fax: +1 301 319 9404.
E-mail address: Madhusoodana.nambiar@na.amedd.army.mil (M.P. Nambiar).

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1. Introduction

Nerve agent and pesticide organophosphate (OP) poisoning represents an ongoing threat to public safety given their low cost and relative ease of synthesis [1–4]. OPs act by irreversible inhibition of acetylcholinesterase (AChE) leading to the build-up of acetylcholine (ACh) at the neuron–neuron and neuro-muscular junction. With the accumulation of ACh, neurons release excitatory amino acids (EAAs), such as glutamate, which bind to the *N*-methyl-D-aspartate (NMDA) receptor causing excitotoxicity and contributes to the seizure/*status epilepticus* (SE) observed with OP toxicity [5]. SE is a long seizure that produces permanent brain damage. Pharmacologic intervention of EAA-induced toxicity at the NMDA receptor is considered a promising approach to protect against seizure/SE and neuropathology [6,7].

Huperzine A (Hup A) is an alkaloid found in the Chinese club moss *Huperzia serrata*. The [−]-Hup A enantiomer is found naturally and [+]-Hup A is synthetic. [−]-Hup A has a much higher affinity for AChE than synthetic [+]-Hup A [8], and it has been demonstrated that [−]-Hup A binds to the bottom of the active site gorge of the enzyme [9]. However, both enantiomers have much higher affinities for AChE than butyrylcholinesterase (BChE) [10], which is being developed as another treatment for nerve agent toxicity [11,12]. Also, [−]-Hup A inhibits both peripheral and central AChE, unlike pyridostigmine bromide, a commonly used OP pretreatment that has been FDA-approved which only inhibits peripheral cholinesterases [13]. This is mainly because Hup A (both enantiomers), unlike pyridostigmine, does not bear a permanent positive charge.

Previous studies have shown that [−]-Hup A has a longer biological half-life than other reversible AChE inhibitors [14] and pre-treatment can prevent seizure. Our group conducted a study in primary guinea pig neuronal cell cultures and demonstrated that Hup A binds to NMDA receptors and protects against glutamate toxicity [15]. Both [+]- and [−]-Hup A were found to antagonize glutamate toxicity (Zang, 2000) although [+]-HupA has 38-fold lower AChE binding activity. [8]. A preliminary study with [+]-Hup A using a radiotelemetry rat seizure/SE model demonstrated that a [+]-Hup A pre-treatment prevents pilocarpine, a muscarinic agonist, induced seizures, but [−]-Hup A does not (data not shown). We hypothesize that [+]-Hup A-induced protection is a result of its *in vivo* NMDA receptor antagonism by blocking its ion channel without inhibiting AChE. The data using the pilocarpine model, *in vitro* Hup A receptor binding experiments [15–18] (Zhang, 2000), and previous voltage clamp experiments [19] led us to determine if the *in vivo* mechanism of action of [+]-Hup A involves NMDA antagonism, and if so, further develop [+]-Hup A as a treatment for seizure and SE induced by toxic OPs.

We used our rat radiotelemetry model to investigate the in vivo mechanism of action induced by [+]-Hup A [20]. Seizures were induced by intracerebroventricular (icv) administration of NMDA, and the animals were treated with different concentrations of [+]-Hup A pre- or post-exposure. We demonstrated that [+]-Hup A prevents NMDA-induced seizure in rats, indicating that its mechanism of action involves NMDA antagonism and blocking EAA-induced toxicity.

2. Materials and methods

2.1. Chemicals

NMDA, tetraisopropyl pyrophosphoramide (*iso*-OMPA), acetylthiocholine iodide (ATCh), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma–Aldrich (St. Louis, MO). [+]-Hup A was obtained from Dr. Robert Moriarty, University of Illinois at Chicago. Isoflurane was purchased from Halocarbon Inc. (River Edge, NJ).

2.2. Animals

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NRC Publication, 1996 edition. Male Sprague–Dawley rats (200–250 g, *Rattus norvegicus*) were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed individually in microisolator cages with a 12 h light/dark cycle. Food and water were available *ad libitum*, and a 1 week stabilization period preceded surgery and experimentation.

2.3. Radiotelemetry

The Radiotelemetry system including 8 receivers and TL10M3-F50-EET bipotential radiotelemetry probes was purchased from Data Sciences International (St. Paul, MN). The probes were sterilized using 4% glutaraldehyde and handled as instructed by the manufacturer.

2.4. Surgical implantation of electroencephalography (EEG) probes

Rats were anesthetized in a chamber with isoflurane gas (2–5% isoflurane, oxygen 1 L/min flow rate). Anesthetized rats were sheared on the head and back and placed in a stereotax (David Kopf Instruments, Tujunga, CA) over a water heating jacket. The mouth and nose of the rat were placed in an adapter connected to a supply of isoflurane gas (2–3% isoflurane, oxygen 1.5 L/min flow rate). The dorsal surfaces of the rat's body and head were cleaned and two small initial incisions were made: one along the midline of the back, 7.5 cm anterior to the tail, and one along the dorsal midline of the head. Two cortical electrodes and a reference electrode were tunneled subcutaneously from the posterior (back) incision to the anterior (head) incision. The skull was cleaned with gauze, and any open veins or arteries were closed by surgical cautery. Three 1 mm burr holes were drilled, and screws were inserted: two screws 3 mm anterior to the lambdoid suture and 3 mm on each side of the sagittal suture, and one screw was inserted 3 mm to the right of the sagittal suture and 3 mm anterior to the coronal suture. The reference electrode was attached