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Assessment of brain oxygenation imbalance following soman exposure in rats

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ABSTRACT

Nerve agents (NAs) are potent organophosphorus (OP) compounds with applications in chemical warfare. OP compounds act by inhibiting acetylcholinesterase (AChE). Soman (*O*-pinacolyl methylphosphonofluoridate) is one of the most potent NAs. It is well known that small doses of NAs can be lethal, and that even non-lethal exposure leads to long-term mental debilitation/neurological damage. However, the neuropathology following exposure to sub-lethal nerve agents is not well understood.

In this study, we examined changes in tissue oxygenation (pO_2) in the cortex and hippocampus after a sub-lethal dose of soman [80–90 μg/kg; subcutaneous]. pO_2 changes can provide information regarding oxygen delivery and utilization and may be indicative of a disruption in cerebral blood flow and/or metabolism. Changes in oxygenation were measured with chronically implanted oxygen sensors in awake and freely moving rats. Measurements were taken before, during, and after soman-induced convulsive seizures.

Soman exposure resulted in an immediate increase in pO_2 in the cortex, followed by an even greater increase that precedes the onset of soman-induced convulsive seizures. The rise in hippocampus pO_2 was delayed relative to the cortex, although the general pattern of brain oxygenation between these two regions was similar. After convulsive seizures began, pO_2 levels declined but usually remained hyperoxygenated. Following the decline in pO_2, low frequency cycles of large amplitude changes were observed in both the cortex and hippocampus. This pattern is consistent with recurring seizures.

Measuring real-time changes in brain pO_2 provides new information on the physiological status of the brain following soman exposure. These results highlight that the measurement of brain oxygenation could provide a sensitive marker of nerve agent exposure and serve as a biomarker for treatment studies.

1. Introduction

Nerve agents (NAs) are potent organophosphorus (OP) compounds with applications in chemical warfare and categorized as weapons of mass destruction. Less potent forms of OPs, including parathion and malathion, are commonly used as pesticides. NAs, which include soman, sarin, tabun, and VX are a significant and current threat to military and civilian populations, because of the potential to cause mass casualties (Ganesan et al., 2010). Despite a ban under the Chemical Weapons Convention Implementation Act, sarin was released in August of 2013 in Syria, killing 1400 people (Dolgin, 2013) and potentially exposing 1000′s to an acute dose.

It is clear that NAs are lethal, however, the pathophysiological neurological changes resulting from a sub-lethal exposure are not well characterized or understood. Repeated acute exposure to OP pesticides has been shown to affect information processing, verbal and visual attention, problem-solving, and motor dexterity (Dassanayake et al., 2007; Rosenstock et al., 1991; Steenland et al., 1994). Studies involving agricultural communities have shown that exposure to long-term low-levels of OP pesticide results in changes to neurological structures in developing children (Rauh et al., 2012). In addition, studies involving victims exposed to NAs have shown changes in regional white matter volume (Chao et al., 2011; Heaton et al., 2007; Yamasue et al., 2007) and grey matter volume (Chao et al., 2011, 2010). Victims of sarin exposure have reported visual, cognitive and motor dysfunction for up to 5 years after poisoning (Kawana et al., 2001). To better understand the neurological effects of NAs at a sub-lethal dose, we exposed rats to soman.

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Soman was selected for its fast action and complexity of treatment, due to the rapid aging of the agent-acetylcholinesterase (AChE) complex (Worek et al., 2004). Initial binding of soman to the AChE active site is a reversible process. However, after 2-2.5 min (Worek et al., 2004) soman undergoes a secondary reaction where the pinacolyl bond is cleaved and permanently binds to the AChE active site (Jokanovic, 2001; Zilker, 2005). Inhibition of AChE results in an accumulation of acetylcholine (ACh) in the synapse causing cholinergic hyperactivity, which leads to a wide range of symptoms, including lacrimation, fasciculation, paralysis, generalized convulsive seizures, and respiratory failure (McDonough and Shih, 1997). Soman also causes generalized convulsive seizures that can potentially develop into a condition known as *status epilepticus* (Lallement et al., 1998; Newmark, 2004; Petras, 1994; Shih and McDonough, 1997), which can last for hours (Koplovitz and Skvorak, 1998). *Status epilepticus* is a life threatening condition defined by seizures lasting more than 5 min or having multiple seizure episodes without recovering consciousness (Lowenstein et al., 1999).

Previous research with soman in animal models has shown seizures to be a contributing factor in neurological damage. In other studies, either through innate tolerance or anti-seizure medication, in the absence of convulsive seizures, no neurological degeneration was observed (Apland et al., 2010; Baille et al., 2005; Guo et al., 2015; Lallement et al., 1993; McDonough et al., 1987). When convulsive seizures were terminated within 20 min, minimal neuronal loss was seen (Baille et al., 2005; Lallement et al., 1993), indicating the presence and duration of seizures to be a critical factor in soman related neurological damage.

Seizures induced by soman (Shih and McDonough, 1997) cause neurological damage through excitotoxicity (Fujikawa et al., 2000; Lallement et al., 1993; Olney et al., 1974). The initiation of seizures is triggered by the excess accumulation of ACh within the synapses (Shih and McDonough, 1997), which in turn causes glutamatergic neurons to release glutamate activating α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Intense activation of AMPA receptors is followed by the activation of *N*-methyl-D-aspartate (NMDA) receptor and enhanced calcium entry. The large influx of calcium results in neurons to be in a state of hyperexcitability, further facilitating the propagation and maintenance of seizures. The intense activation of NMDA receptors can activate a cell death pathway, leading to neuronal cell death (Fujikawa et al., 2000; Lallement et al., 1993).

Measurements of pO_2 provide information about metabolic imbalance in the brain, which in turn can lead to neuronal damage. By directly measuring pO_2, we can get an indication of the balance between oxygen delivery (cerebral blood flow or CBF) and cerebral metabolic rate of oxygen ($CMRO_2$). We developed a method to measure regional pO_2 in the brain using chronically implanted fiber-optic oxygen sensors in awake and freely moving rats (Ortiz-Prado et al., 2010). This method allows for the measurement of pO_2 without anesthesia or other stresses (handling or restraint) that can potentially alter related physiological responses.

The objective of this study was to measure pO_2 in the rat brain after soman-induced convulsive seizures. This paper is the first to measure pO_2 changes before, during, and after soman-induced convulsive seizures in awake and freely moving rats. Brain oxygenation was directly measured in the cortex and hippocampus, which are areas selected for their sensitivity to NA related neurological damage (Abdel-Rahman et al., 2002; Petras, 1994).

2. Materials and methods

2.1. Animals

Animal care protocols were approved by the University of Calgary Animal Care Committee and meet the Canadian Council of Animal Care (CCAC) guidelines. Twelve male Sprague-Dawley rats were obtained from Charles River Laboratories (Montréal, QC, Canada) weighing 200–350 g. Rats were housed in the University of Calgary Animal Care Facility (Calgary, AB, Canada) with a 12-h light/dark cycle. Each cage housed two rats until probe implantation. Rats were handled for an additional 2–3 days to acclimate to human touch. Surgical implantation of probes was completed following acclimation. Following the probe implantation, rats were housed in individual cages with access to food and water *ad libitum*. Rats were closely monitored on a daily basis by staff for general health status.

2.2. pO_2 probe implantation

Fiber-optic probes were implanted in the cortex and hippocampus via microsurgical methods. The fiber lengths were 4 mm for the hippocampus and 3 mm for the cortex. Animals were anesthetized with isoflurane via inhalation prior to surgery and maintained on 70% N_2, 30% O_2, and 2% isoflurane during the surgical procedure. Temperature and respiration rate were monitored and maintained over the course of implantation. Scalp was shaved and sterilized with iodine solution, a midline incision was made and the skin was retracted laterally to expose the skull. Holes were drilled through the skull at stereotaxic coordinates relative to the bregma. Cortex coordinates: +1 mm anterior/posterior, +1.5 mm medial/lateral, and −2 mm from the top of the skull. Hippocampus coordinates: −4 mm anterior/posterior, −3.5 mm medial/lateral, and −3 mm from the top of the skull. To further secure the probes, 3 additional holes were drilled and implanted with plastic screws. The probes and screws were secured with dental cement and molded into a head cap. The retracted skin was secured to the head cap with cyanoacrylate glue. Rats were administered buprenorphine (0.1 mg/kg; subcutaneous) post-surgery for analgesic control. Rats were monitored closely during surgical recovery to ensure the head cap was secure and discomfort was minimized. Rats were given two doses of buprenorphine (0.1 mg/kg) per day for up to three days post-surgery and were monitored minimum of twice daily for general health status and any signs of pain or stress.

2.3. Drugs

Soman (CAS 96-64-0) was diluted in isopropyl alcohol (Sigma-Aldrich, Millwaukee, WI, USA) and then sterile saline (0.9% NaCl, Baxter, Canada) to the maximum concentration required for the heaviest rat used on the day of exposure. A dose of 80–90 μg/kg was used in this study based on a pilot study that showed this dose consistently resulted in convulsive seizures. This dose is 0.72–0.82xLD50 based on a published LD50 of 110 μg/kg subcutaneous (Shih et al., 1990).

The oxime, HI-6 dimethanesulfonate (CAS 144252-71-1) was provided by the Defence Research and Development Canada (DRDC) Suffield Research Centre. A dose of 125 mg/kg was prepared in 0.9% sterile saline. The same dose of HI-6 was used pre- and post soman injection.

Atropine Methyl Nitrate (AMN) was purchased from Sigma Aldrich (Milwaukee, WI, USA). A dose of 20 mg/kg of AMN was prepared in 0.9% sterile saline. The same dose of AMN was used pre- and post soman injection.

2.4. Magnetic resonance imaging

Rats were imaged after probe implantation to confirm the relative probe location and check for intracranial bleeding 24 h after surgery. Imaging was done with a 9.4T MRI and a Bruker Avance console (Bruker Biospin GmbH, Rheinstetten, Germany).