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Repeated exposure to chlorpyrifos leads to prolonged impairments of axonal transport in the living rodent brain

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ABSTRACT

The toxicity of the class of chemicals known as the organophosphates (OP) is most commonly attributed to the inhibition of the enzyme acetylcholinesterase. However, there is significant evidence that this mechanism may not account for all of the deleterious neurologic and neurobehavioral symptoms of OP exposure, especially those associated with levels that produce no overt signs of acute toxicity. In the study described here we evaluated the effects of the commonly used OP-pesticide, chlorpyrifos (CPF) on axonal transport in the brains of living rats using manganese (Mn^{2+})-enhanced magnetic resonance imaging (MEMRI) of the optic nerve (ON) projections from the retina to the superior colliculus (SC). T1-weighted MEMRI scans were evaluated at 6 and 24 h after intravitreal injection of Mn^{2+}. As a positive control for axonal transport deficits, initial studies were conducted with the tropolone alkaloid colchicine administered by intravitreal injection. In subsequent studies both single and repeated exposures to CPF were evaluated for effects on axonal transport using MEMRI. As expected, intravitreal injection of colchicine (2.5 μg) produced a robust decrease in transport of Mn^{2+} along the optic nerve (ON) and to the superior colliculus (SC) (as indicated by the reduced MEMRI contrast). A single subcutaneous (s.c.) injection of CPF (18.0 mg/kg) was not associated with significant alterations in the transport of Mn^{2+}. Conversely, 14-days of repeated s.c. exposure to CPF (18.0 mg/kg/day) was associated with decreased transport of Mn^{2+} along the ONs and to the SC, an effect that was also present after a 30-day (CPF-free) washout period. These results indicate that repeated exposures to a commonly used pesticide, CPF can result in persistent alterations in axonal transport in the living mammalian brain. Given the fundamental importance of axonal transport to neuronal function, these observations may (at least in part) explain some of the long term neurological deficits that have been observed in humans who have been repeatedly exposed to doses of OPs not associated with acute toxicity.

1. Introduction

The chemicals known as the organophosphates (OPs) are used for a wide variety of important applications and they are especially prevalent in the agricultural setting where they have been applied as pesticides for decades. Unfortunately, OPs are highly toxic to humans as well as target organisms and continuing reports of accidental and intentional poisonings (i.e., from suicide attempts) by OPs is an ongoing environmental and public health concern worldwide (reviewed, Eddleston et al., 2008). The risk of exposure to OP-based nerve agents from rogue governments and terrorist organizations is an additional threat that was recently exemplified by the sarin attacks on civilians in Syria (United Nations Security Council Report, 2013).

The toxic "cholinergic crisis" associated with acute poisoning with OPs and the associated variety of long term neurologic and neurobehavioral consequences have been studied extensively and are primarily attributed to the inhibition of acetylcholinesterase (AChE) (Ecobichon, 2001, for review see also Pereira et al., 2014), However, there is also significant evidence in the human epidemiological literature (e.g., Ross et al., 2013) that OP exposures not associated with acute toxicity may also result in prolonged neurological and neurobehavioral deficits including impairments of cognition. Moreover, as an etiological mechanism, AChE

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inhibition may not account all of the symptoms associated with acutely toxic or lower level OP exposures as suggested by the following lines of evidence (reviewed in Banks and Lein, 2012): (1) different OPs can have different toxicological profiles despite having similar effects on AChE activity (Bushnell and Moser, 2006; Jett and Lein, 2006; Pope et al., 2005; Pope, 1999), (2) the OP nerve agent, VX induced neurotoxic effects in AChE knockout mice (Duysen et al., 2001); (3) reports in both the human and animal literature indicate that OP toxicity (especially associated with chronic exposure) can occur in the absence of AChE inhibition (Abou-Donia, 2003; Costa, 2006; Kamel and Hoppin, 2004); (4) human studies of occupational exposures to OPs often fail to find a significant correlation between blood cholinesterase activity and neurobehavioral deficits (Rohlman et al., 2011).

Prospective efforts to further elucidate the long term consequences of OP exposures as well as the mechanisms of the deleterious neurological effects require the use of animals and other model systems. Interestingly, more than 30 years ago experiments in animals indicated that axonal transport is negatively affected by OPs, a potentially notable finding given the fundamental importance of axonal transport to neuronal health and brain function. In these early experiments, relatively high doses of phenylphosphonothioate esters and tri-o-cresyl phosphate (i.e., compounds associated with OP-induced delayed neuropathies-OPIDN) inhibited fast anterograde axonal transport in an ex vivo rat optic nerve preparation (Reichart and Abou-Donia, 1980). Later studies in our laboratories indicated that both anterograde and retrograde transport of vesicles in the sciatic nerves (ex vivo) was impaired in rats repeatedly exposed to chlorpyrifos (O,O-diethyl O-[3,5,6,-trichloro-2-pyridyl] phosphorothionate) (CPF), an insecticide OP not associated with OPIDN except at doses well above the LD_{50} (see Richardson, 1995). In these studies doses were used that were not associated with signs of acute toxicity, and further, the axonal transport deficits persisted after a 14-day CPF-free washout period (Terry et al., 2003). Using the same experimental approach, later time course studies indicated that a significant reduction in axonal transport occurred within 10 h of a single CPF exposure (18.0 mg/kg s.c.) (Terry et al., 2007).

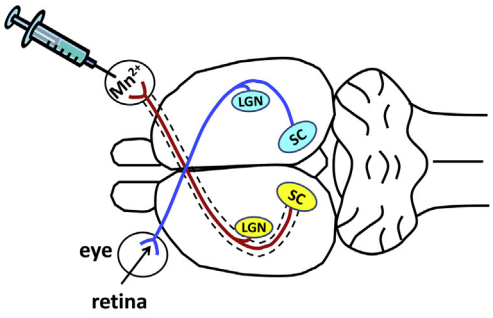
The purpose of the study described here was evaluate the effects of CPF on axonal transport in the brains of living rats using manganese (Mn^{2+})-enhanced magnetic resonance imaging (MEMRI), a non-invasive imaging method that has gained popularity in the last few years. Thus far, the technique has been successfully used to detect impairments of axonal transport in the brains of aged rats (Cross et al., 2008), mouse models of Alzheimer's disease (Kim et al., 2011; Smith et al., 2007, 2011), frontotemporal dementia (Majid et al., 2014), and mice homozygous for a deletion in the amyloid precursor protein gene (Gallagher et al., 2012). MEMRI exploits both the paramagnetic properties of Mn^{2+} and its ability to serve as a calcium analog in neurons. Due to its paramagnetic properties, Mn^{2+} shortens the longitudinal relaxation time constant, T1, of neighboring water, leading to increased intensity in T1-weighted images that can be detected (Lin and Koretsky, 1997) and tracked dynamically over time (Pautler et al., 1998; Pautler and Koretsky, 2002). As a calcium analog, Mn^{2+} enters neurons via voltage-gated calcium channels (Drapeau and Nachshen, 1984; Narita et al., 1990; Sloot and Gramsbergen, 1994; Lu et al., 2007), where it travels within vesicles along microtubules by fast axonal transport (Merritt et al., 1989; Takeda et al., 1998; Silva et al., 2004; Smith et al., 2007; Zhang et al., 2010) in a process that is at least partially dependent on the motor protein kinesin (Bearer et al., 2007, 2009). Here we utilized MRI to visualize Mn^{2+} enhancement of the optic nerve projections (see Lin et al., 2014) from the retina to the superior colliculus. From intravitreal injection, Mn^{2+} has been shown to enter retinal ganglion cells and to travel within their axons in the anterograde direction along the optic nerve to the superior colliculus and lateral geniculate nucleus (Bearer et al., 2007; Watanabe et al., 2001). As a positive control for axonal transport deficits, initial studies were conducted with colchicine administered by intravitreal injection. In subsequent studies, both a single exposure and repeated exposures to CPF administered by subcutaneous injection were evaluated. After the repeated exposure experiments, an extended OP-free washout period was also assessed.

2. Materials and methods

A diagram illustrating the intravitreal injection method and the transport of Mn^{2+} within the axons of retinal ganglion cells in the anterograde direction along the optic nerve to the contralateral superior colliculus and lateral geniculate nucleus is presented in Fig. 1 as well as an overview of the three MEMRI studies described in this report (additional details are provided below).

2.1. Animals

Male albino Wistar rats (Harlan, Indianapolis, IN, USA) 2–3 months old ($n = 6$ per test group) were housed in pairs in a temperature controlled room (25 °C), maintained on a standard 12-h light/dark cycle with free access to food (Teklad Global



Study	Procedure	Imaging	
		6 hrs	24 hrs
1: Colchicine Validation	Mn^{2+} + Colchicine Combined Intravitreal Injection	MRI	MRI
2: CPF Single Expoure	Mn^{2+} Intravitreal Injection CPF s.c. Injection	MRI	MRI
3: CPF Repeated Exposure	Baseline Evaluation Mn^{2+} Intravitreal Injection	MRI	MRI
	↓ CPF s.c. Injection One per day x 14 days		
	↓ 24 hr CPF-Free Washout Mn^{2+} Intravitreal Injection	MRI	MRI
	↓ 30 day CPF-Free Washout Mn^{2+} Intravitreal Injection	MRI	MRI

Fig. 1. Diagram illustrating the intravitreal injection method used in this study and the transport of Mn^{2+} within the axons of retinal ganglion cells (retina) in the anterograde direction (red line) along the optic nerve to the contralateral superior colliculus (SC) and lateral geniculate nucleus (LGN). Below the diagram is the experimental design for each of the three studies described in this report. Abbreviations: chlorpyrifos (CPF), manganese (Mn^{2+}), subcutaneous (s.c.), hours (hrs), magnetic resonance imaging (MRI).