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Comparative effects of parathion and chlorpyrifos on endocannabinoid and endocannabinoid-like lipid metabolites in rat striatum

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ABSTRACT

Parathion and chlorpyrifos are organophosphorus insecticides (OPs) that elicit acute toxicity by inhibiting acetylcholinesterase (AChE). The endocannabinoids (eCBs, N-arachidonoylethanolamine, AEA; 2-arachidonoylglycerol, 2AG) are endogenous neuromodulators that regulate presynaptic neurotransmitter release in neurons throughout the central and peripheral nervous systems. While substantial information is known about the eCBs, less is known about a number of endocannabinoid-like metabolites (eCBLs, e.g., N-palmitoylethanolamine, PEA; N-oleoylethanolamine, OEA). We report the comparative effects of parathion and chlorpyrifos on AChE and enzymes responsible for inactivation of the eCBs, fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), and changes in the eCBs AEA and 2AG and eCBLs PEA and OEA, in rat striatum. Adult, male rats were treated with vehicle (peanut oil, 2 ml/kg, sc), parathion (27 mg/kg) or chlorpyrifos (280 mg/kg) 6–7 days after surgical implantation of microdialysis cannulae into the right striatum, followed by microdialysis two or four days later. Additional rats were similarly treated and sacrificed for evaluation of tissue levels of eCBs and eCBLs. Dialysates and tissue extracts were analyzed by LC–MS/MS. AChE and FAAH were extensively inhibited at both time-points (85–96%), while MAGL activity was significantly but lesser affected (37–62% inhibition) by parathion and chlorpyrifos. Signs of toxicity were noted only in parathion-treated rats. In general, chlorpyrifos increased eCB levels while parathion had no or lesser effects. Early changes in extracellular AEA, 2AG and PEA levels were significantly different between parathion and chlorpyrifos exposures. Differential changes in extracellular and/or tissue levels of eCBs and eCBLs could potentially influence a number of signaling pathways and contribute to selective neurological changes following acute OP intoxications.

1. Introduction

Parathion and chlorpyrifos are both organophosphorus insecticides (OPs). Parathion is no longer registered for use in the US, but remains in use in many other countries. Chlorpyrifos is one of the most commonly used OPs in the United States and throughout the world (Grube et al., 2011). OPs elicit acute toxicity through a common mechanism initiated by acetylcholinesterase (AChE) inhibition (Mileson et al., 1998). Extensive inhibition of AChE leads to elevated levels of acetylcholine in cholinergic synapses throughout the body and consequent cholinergic signs of toxicity including tremors, excessive parasympathetic end organ secretions (e.g., lacrimation), vomiting, miosis and various other signs and symptoms (Pope et al., 2005; Wilson, 2010).

Endocannabinoids (eCBs, e.g., arachidonoylethanolamine [AEA] and 2-arachidonoylglycerol [2AG]) are global neuromodulators produced from membrane lipids and released by postsynaptic neurons following depolarization, as well as through receptor-mediated signaling through cholinergic muscarinic M1 and M3 and other neurotransmitter receptors (Castillo et al., 2012). Once released, the eCBs diffuse across the synapse and activate cannabinoid (CB1) receptors on the presynaptic neuron terminal. CB1 activation generally leads to the inhibition of neurotransmitter release in a wide variety of neurons and signaling pathways, in both the central and peripheral nervous systems. The other primary cannabinoid receptor, CB2, is more prominently located on immune cells with lesser putative role in neuromodulation. While the neuromodulatory role of AEA and 2AG has been well

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documented, a number of eCB-like lipid metabolites (eCBLs) have been more recently reported to have effects in the nervous system (see review of Fezza et al., 2014). The eCBLs (e.g., N-palmitoylethanolamine, PEA; N-oleolyethanolamine, OEA) have no direct effects on CB1 or CB2 receptors but can activate other macromolecular receptors including the peroxisome proliferator-activated receptors and/or the orphan G-protein coupled receptors GPR55 and GPR119 (LoVerme et al., 2005; Godlewski et al., 2009; Hansen, 2010; Pistis and Melis, 2010; Liu et al., 2015).

Disruption of acetylcholine hydrolysis and consequent elevation of synaptic acetylcholine levels is *sine qua non* for the development of cholinergic toxicity following OP exposure (DuBois et al., 1949). As eCBs can decrease acetylcholine release via activation of CB1 receptors (Gifford and Ashby, 1996; Gifford et al., 2000; Tzavara et al., 2003; Degroot et al., 2006), and CB1 antagonists can increase acetylcholine release (Gifford and Ashby, 1996; Kathmann et al., 2001), we proposed that eCBs can play a role in OP toxicity. Indeed, we previously reported that acute toxicity of both paraoxon (the active metabolite of parathion) and diisopropylfluorophosphate, is reduced by CB1 receptor agonists (Nallapaneni et al., 2006, 2008; Wright et al., 2010). More recently, the CB1 receptor antagonist AM251 was shown to increase the acute toxicity of paraoxon and chlorpyrifos oxon (the active metabolite of chlorpyrifos; Liu and Pope, 2015).

The enzymatic degradation of AEA is primarily mediated by the enzyme fatty acid amide hydrolase (FAAH, Cravatt et al., 1996, 2001; Egertova et al., 2003). FAAH is also critical in the breakdown of the eCBLs PEA and OEA. While the primary and often most sensitive macromolecular target for many OPs is AChE, a number of OPs including parathion and chlorpyrifos (in vivo) and paraoxon and chlorpyrifos oxon (in vitro) are potent inhibitors of FAAH (Quistad et al., 2001, 2006; Nomura et al., 2008; Nallapaneni et al., 2006, 2008; Liu et al., 2013) and can elevate brain levels of AEA (Nomura et al., 2008; Carr et al., 2013). Monoacylglycerol lipase (MAGL) is the main enzyme involved in 2AG hydrolysis (Blankman et al., 2007; Hashimotodani et al., 2007; Savinainen et al., 2012). MAGL is also inhibited by a number of OPs but, in general, MAGL is less sensitive than FAAH to inhibition by OPs (Quistad et al., 2001, 2006; Nomura et al., 2008; Nomura and Casida, 2011; Liu et al., 2013). OP-mediated inhibition of MAGL has been reported to increase brain levels of 2AG in mice and rats (Nomura et al., 2008; Nomura and Casida, 2011; Carr et al., 2013). We previously reported that in vivo exposure to either parathion or chlorpyrifos led to similar degrees of inhibition in both FAAH and MAGL activities in the hippocampus, with concomitant increases (more so with chlorpyrifos) in extracellular AEA levels. Hippocampal 2AG level was only increased by chlorpyrifos, however (Liu et al., 2013).

In this study, we evaluated the effects of high in vivo dosages of parathion and chlorpyrifos on rat striatal AChE, FAAH and MAGL, and both extracellular and tissue levels of AEA, 2AG, PEA and OEA. As eCBs and eCBLs can be involved in a variety of processes including neuromodulation, gene expression, inflammation and others, OP- and regional-selective changes in eCBs and eCBLs could potentially contribute to cholinergic and non-cholinergic mechanisms involved in acute and long-term consequences of OP intoxication.

2. Methods

2.1. Chemicals and reagents

Parathion (O,O′-diethyl-*p*-nitrophenyl-phosphorothioate) and chlorpyrifos (O,O′-diethyl-3,5,6-trichloro-2-pyridinyl-phosphorothioate), >99% pure; GC/MS analysis, were purchased from ChemService (West Chester, PA). Acetylcholine iodide (acetyl-^{3}H, specific activity 76.0 Ci/mmol) was purchased from Perkin Elmer (Wellesley, MA). [^{3}H]Anandamide (ethanolamine 1-^{3}H) specific activity 60 Ci/mmol, was purchased from American Radiolabeled Chemicals (St. Louis, MO). Arachidonoyl-1-thio-glycerol and anandamide were purchased from Cayman Chemical (Ann Arbor, MI). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO). Guide cannulae (MD 2250) and microdialysis probes (MD 2204, 4 mm membrane) were purchased from Bioanalytical Systems Inc. (BAS, West Lafayette, IN).

2.2. Animals and treatments

Male, Sprague–Dawley rats (approximately 3 months of age) were purchased from Harlan (Indianapolis, IN) and maintained in the AAALAC-accredited Animal Resources facility at Oklahoma State University. Animals were housed in polycarbonate cages with a 12-h:12-h light:dark cycle, allowed free access to food (PMI® Laboratory Rodent Diet 5001, PMI Feeds, Richmond, IN) and water throughout, and acclimated to the facility for 5–7 days prior to beginning the study. Surgical procedures and routine animal care were in accordance with protocols established in the NIH/NRC *Guide for the Care and Use of Laboratory Animals* and approved by the local Institutional Animal Care and Use Committee.

OP compounds were dissolved in peanut oil (100% pure; Lou-Ana brand, Ventura Foods, Opelousas, LA) and injected subcutaneously (sc) at a volume of 2 ml/kg. Rats were treated with vehicle, parathion (27 mg/kg) or chlorpyrifos (280 mg/kg). Functional signs of toxicity were recorded essentially as described earlier (Liu et al., 2013) by a trained observer "blinded" to treatment groups. Autonomic signs (i.e., SLUD, an acronym for salivation, lacrimation, urination and defecation) were graded as: 1 = normal (no secretions); 2 = mild one or multiple secretions; 3 = moderate multiple secretions; 4 = severe multiple secretions. Involuntary movements were scored as: 2 = normal quivering of vibrissae and head; 3 = fine head and neck tremors; 3.25 = more consistent tremors in head, neck and forelimbs; 3.5 = consistent tremors extending caudally from head to the midbody; 3.75 = tremors extending caudally to the hindlimbs; 4 = whole body tremors; 5 = myoclonic jerks.

2.3. Stereotaxic surgery, microdialysis and metabolite analysis

A guide cannula was surgically implanted into the right striatum. Animals were first anesthetized with a ketamine/xylazine (9:1) mixture (0.6 ml/kg, ip). The scalp was shaved and the head was positioned into a stereotaxic apparatus (Stoelting Co., Wood Dale, IL). The cannula was positioned using the coordinates: anterior–posterior, 1.2 mm, medial–lateral, −2.2 mm; dorsal–ventral, -3.4 mm from bregma. Two screws were placed on each side of the cannula and dental cement was used to secure the cannula. Animals were allowed to recover for 6–7 days prior to OP treatment.

On the day of microdialysis, rats were lightly anesthetized with isoflurane and the probe was rapidly inserted into the guide cannula. Rats were then transferred into a Raturn® animal chamber (BAS, West Lafayette, IN) and dialysis tubing was stabilized with a plastic collar. The dialysis probe was equilibrated for 5 h by perfusion with artificial cerebrospinal fluid (aCSF: NaCl, 149 mM; KCl, 2.8 mM; $CaCl_2$, 1.2 mM; $MgCl_2$, 1.2 mM; ascorbic acid, 0.25 mM; D-glucose, 5.4 mM; hydroxypropyl-β-cyclodextrin, 30% [to increase eCB capture, Caillé et al., 2007]) at flow rate of 0.8 μl/min using a syringe pump (MD 1101, BAS). Following equilibration, five fractions (15 min each) were collected into a refrigerated fraction collector. All fractions were stored at −80 °C until analysis. Cannula/probe placement was verified in all tissues by H&E staining.