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Differential expression of glutamate transporters in cerebral cortex of paraoxon-treated rats

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ABSTRACT

Glutamatergic system is involved in pathological effects of organophosphorus (OP) compounds. We aimed to determine in vivo effects of paraoxon, the bioactive metabolite of parathion, on the expression of glutamate transporters as well as Bax and Bcl2 in rat cerebral cortex. Male Wistar rats received an intraperitoneal (i.p.) injection of one of three doses of paraoxon (0.3, 0.7, or 1 mg/kg) or corn oil as vehicle (1 ml/kg). After 4 or 18 h, cerebral cortices were dissected out and used for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and western blot assays to measure mRNA and protein levels, respectively. The cortical glial glutamate transporters (GLAST and GLT-1) were up-regulated in animals treated with 0.7 mg/kg of paraoxon, but down-regulated in 1 mg/kg group. Neuronal glutamate transporter (EAAC1) was unchanged in 0.7 mg/kg treated rats, while reduced in 1 mg/kg group. No significant difference was found in the mRNA and protein expression of EAAC1 in animals intoxicated with 0.3 mg/kg of paraoxon. Paraoxon (1 mg/kg) resulted in an up-regulation of Bax and down-regulation of Bcl2 mRNA levels in the rat cerebral cortex. These results indicate that paraoxon can differentially regulate expression of glutamate transporters at mRNA and protein levels in the cerebral cortex. Changes in the expression of glutamate transporters are closely related to paraoxon-induced seizure activity.

1. Introduction

Organophosphorus (OP) compounds refer to a large group of insecticides or nerve agents, which act by inhibiting the enzyme acetylcholinesterase (AChE), the enzyme responsible for the breakdown of acetylcholine. Subsequently, accumulation of acetylcholine (ACh) leads to hyperstimulation of muscarinic and nicotinic receptors and produces a series of centrally and peripherally pathological responses, including hypersecretions, fasciculation, tremor, convulsions, respiratory distress, and death (Shih et al., 2003).

Poisoning with pesticides, especially OP insecticides, is a major public-health concern worldwide (Albuquerque et al., 2006; Buckley et al., 2004). OP insecticide parathion is converted to its bioactive metabolite paraoxon by oxidative desulforation in the liver. Paraoxon is used in civilian laboratories as a surrogate nerve agent (Deshpande et al., 2014).

Although the primary known mechanism of OP action is disruption in acetylcholine neurotransmission, these compounds have also been reported to interfere with other neurotransmitter systems, including GABAergic and glutamatergic systems. Current standard treatments for reducing OP-induced toxicity include anticholinergic compounds to reduce the muscarinic syndrome, oximes to reactivate inhibited AChE, and anticonvulsants to control OP-induced seizures. However, such treatments failed to prevent long-term OP-induced seizures and subsequent brain damages (Guo et al., 2015; Shih et al., 2003). Therefore, research efforts are necessary in order to identify more efficient drugs to provide neuroprotection against OP-induced brain damages. Modulation of the glutamatergic system and reduction of glutamate excitotoxicity appear to be one of the therapeutic strategies to prevent neuronal death and consequent cognitive impairment caused by OP-induced seizure (Myhrer et al., 2005).

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system and essential for normal brain function including cognition, memory, and learning. However, the extracellular concentration of glutamate must remain below its excitotoxic levels to limit overstimulation of glutamate receptors and prevent neuronal damage or death (Danbolt, 2001). In addition to the amount of glutamate released, the concentration of glutamate in the synaptic cleft is

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determined by its clearance through high affinity sodium dependent glutamate transporters, also called excitatory amino acid transporters, EAATs. These transporters include five members: EAAT1 (glutamate-aspartate transporter, GLAST), EAAT2 (glutamate transporter-1, GLT-1), EAAT3 (excitatory amino acid carrier 1, EAAC1), EAAT4, and EAAT5. EAAT1 and EAAT2 are mainly expressed in astrocytes, whereas EAAT3 is expressed in neurons. EAAT4 and EAAT5 appear to be restricted to cerebellar Purkinje cells and the retina, respectively (Danbolt, 2001; Simantov et al., 1999; Zhou and Danbolt, 2013). These transporters are responsible for extracellular glutamate clearance by glutamatergic axon terminals and astrocytic processes (Danbolt, 2001). Astrocytic glutamate transporters, especially EAAT2, have the main role in clearing glutamate from the extracellular space (Danbolt, 2001; Rothstein et al., 1996).

By regulation of synaptic glutamate concentration, glutamate transporters play an important role in limiting glutamate signaling and controlling the activation of glutamate receptors (Rimmele and Rosenberg, 2016). Reduced glutamate transport is thought to underlie the pathogenesis of numerous neurological diseases, such as epilepsy (Coulter and Eid, 2012), amyotrophic lateral sclerosis (ALS) (Maragakis and Rothstein, 2001), and Alzheimer's disease (Scimemi et al., 2013). For example, a significant loss of EAAT2 protein has been found in ALS, in which down-regulation of the transporter is associated with neurodegeneration (Maragakis and Rothstein, 2001). Factors that affect glutamate transporter expression and activity can lead to alterations in excitatory neurotransmission (Rothstein et al., 1995; Shashidharan et al., 1994).

The results obtained from our previous studies support the hypothesis that exposure to OP compounds can lead to alterations in the brain GABAergic and glutamatergic systems (Ghasemi et al., 2007; Mohammadi et al., 2008; Mohammadi et al., 2016). Whereas evidence for recruitment of glutamatergic mechanisms after OP intoxication is well known, the effect of OP compounds on glutamate transporters has not been investigated in detail. Recently, we studied alterations in the expression of the glutamate transporters in the rat hippocampus following administration of paraoxon (Mohammadi et al., 2016). Significant increases in the mRNA and protein levels of both glial glutamate transporters were found in rats treated with convulsive doses of paraoxon. Since cerebral cortex is rich in cholinergic and glutamatergic innervations and long-lasting excitability spreads to cortical areas, we thus hypothesized that exposure to paraoxon could cause alterations in cerebral glutamatergic system. In the present study, the expression of glutamate transporters was measured in the cerebral cortex of paraoxon-treated rats at mRNA and protein levels.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were purchased from Sigma-Aldrich Company (U.K.) unless otherwise indicated.

2.2. Animal care and treatments

All procedures were carried out in accordance with standards for care and use of laboratory animals approved by Ethical Committee of the Mazandaran University of Medical Sciences, Sari, Iran. Male Wistar rats weighing 200–250 g were housed four per cage in a controlled environment with temperature of 23 ± 2 °C and a 12 h dark/light cycle. Animals had free access to food and water.

Animals received an intraperitoneal (i.p.) injection of one of three doses of paraoxon (O,O-diethyl O-(4-nitrophenyl) phosphate) dissolved in corn oil (0.3, 0.7, or 1 mg/kg) or vehicle (corn oil). Solutions were administered shortly after preparation (1 ml/kg). After 4 or 18 h, the animals were sacrificed, brains were immediately removed, and cerebral cortices were dissected out and stored at − 70 °C until use; one for mRNA and the other for protein determination. Another set of animals was used for AChE assay. The doses of paraoxon and exposure time-points were selected based on previous studies (Coudray-Lucas et al., 1984; Mohammadi et al., 2016). Five animals were used for each treatment group and time point.

2.3. AChE assay

Whole cerebral cortex from each animal was removed and homogenized with 1% Triton X-100 in 0.1 M sodium phosphate buffer. AChE activity was immediately measured using a modified method of Ellman et al. (Ellman et al., 1961) as previously described (Mohammadi et al., 2008). Protein concentration was determined by the method of Bradford (Bradford, 1976). The AChE activity was expressed as nanomoles of substrate hydrolyzed/min/mg protein.

2.4. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR)

Total RNA was extracted from dissected tissues using a commercial RNA extraction kit (RNeasy Minikit, Qiagen, Germany), according to the manufacturer's instructions. The quantity and quality of extracted RNAs were assessed by a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA) and agarose gel electrophoresis, respectively. 1 µg of total RNA was used to generate cDNA using the RevertAid™ First-Strand cDNA synthesis kit (Thermo Fisher Scientific) primed with random hexamer primer as the manufacturer's instructions.

Primers for amplification of glutamate transporters (GLAST, GLT-1,

Table 1
Primers used for qRT-PCR.

Gene	Primers (5′-3′)[a]	Product Size (bp)	Accession number
GLAST	For. GGA CCT CCT CAA GTT CTG Rev. GTG GCT GTG ATA CTT ATT GTT A	220	NM_019225.2
GLT-1	For. TGT TTC CAA CAG ATT CAG ACT G Rev. ATC CAA TCA GAC CTA AGA CAT TC	190	NM_017215.2
EAAC1	For. CAA CAA TGT CTG AGA ACA AGA Rev. TGT AAC ACA TGA TGA TCT GAA C	204	NM_013032.3
Bax	For. GGC TGG ACA CTG GAC TTC Rev. CAG ATG GTG AGT GAG GCA	152	NM_017059.2
Bcl2	For. GTG GAC AAC ATC GCT CTG Rev. AGA CAG CCA GGA GAA ATC A	141	NM_016993.1
HGPRT	For. CAG CGT CGT GAT TAG TGA Rev. GGT CAG CAA AGA ACT TAT AGC	215	NM_000194.2

[a] For., Forward primer; Rev., Reverse primer.