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Research Report

Neuroprotective mechanisms activated in non-seizing rats exposed to sarin

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ABSTRACT

Exposure to organophosphate (OP) nerve agents, such as sarin, may lead to uncontrolled seizures and irreversible brain injury and neuropathology. In rat studies, a median lethal dose of sarin leads to approximately half of the animals developing seizures. Whereas previous studies analyzed transcriptomic effects associated with seizing sarin-exposed rats, our study focused on the cohort of sarin-exposed rats that did not develop seizures. We analyzed the genomic changes occurring in sarin-exposed, non-seizing rats and compared differentially expressed genes and pathway activation to those of seizing rats. At the earliest time point (0.25 h) and in multiple sarin-sensitive brain regions, defense response genes were commonly expressed in both groups of animals as compared to the control groups. All sarin-exposed animals activated the MAPK signaling pathway, but only the seizing rats activated the apoptotic-associated JNK and p38 MAPK signaling sub-pathway. A unique phenotype of the non-seizing rats was the altered expression levels of genes that generally suppress inflammation or apoptosis. Importantly, the early transcriptional response for inflammation- and apoptosis-related genes in the thalamus showed opposite trends, with significantly down-regulated genes being up-regulated, and vice

Abbreviations: 2-PAM, 2-pyridine aldoxime methylchloride; ACh, acetylcholine; AChE, acetylcholinesterase; Avp, arginine vasopressin; Bcat1, branched chain amino-acid transaminase; Btg2, B-cell translocation gene 2; Dbp, D site of albumin promoter binding protein; DEG, differentially expressed gene; Dusp1, dual specificity phosphatase 1; Ephx2, epoxide hydrolase 2; FC, $\log_2$ fold-change; FDR, false discovery rate; Gja1, gap junction protein, α1; GO, Gene Ontology; Homer1, Homer protein homolog; Ier2, immediate early response 2; Ilf3, interleukin enhancer binding factor 3; KEGG, Kyoto Encyclopedia of Genes and Genomes; NMDA, N-methyl-D-aspartate; Nqo2, NAD(P)H/quinone dehydrogenase 2; OP, organophosphate; Plekhb2, pleckstrin homology domain containing, family B member 2, evectins; Ppp3r1, protein phosphatase 3, regulatory subunit B, alpha isoform, calcineurin B type I; Prim1, DNA primase, p49 subunit; Prkacb, protein kinase, cAMP-dependent, catalytic, beta; Retsat, retinol saturase; Rnf6, ring finger protein 6, C3H2C3 type; sEH, soluble epoxide hydrolase 2; Ttr, transthyretin; Zeb2, zinc finger E-box binding homeobox 2; Zfp36, zing finger protein 36

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versa, between the seizing and non-seizing rats. These observations lend support to the hypothesis that regulation of anti-inflammatory genes might be part of an active and sufficient response in the non-seizing group to protect against the onset of seizures. As such, stimulating or activating these responses via pretreatment strategies could boost resilience against nerve agent exposures.

1. Introduction

Highly toxic organophosphate (OP) nerve agents, such as sarin (O-isopropyl methylphosphonofluoridate), inhibit acetylcholinesterase (AChE), an enzyme that hydrolyzes and regulates the neurotransmitter acetylcholine (ACh). The inactivation of AChE causes a toxic buildup of postsynaptic ACh and results in the overstimulation of muscarinic and nicotinic ACh receptors (McDonough and Shih, 1997). Systemic damage follows a three-phase neuropharmacological process in which 1) the initial hyperstimulation of the cholinergic receptors by excess ACh initiates seizures, followed by 2) a predominantly glutamatergic phase that sustains the seizure, and 3) a final state where the excessive stimulation of ionotropic glutamate receptors causes excessive elevations in intracellular sodium and calcium concentrations. This imbalance of ions, especially the increase in intracellular free calcium, produces a harmful cascade of pathological processes leading to excitotoxic cell death (McDonough and Shih, 1997). Seizure duration and intensity are correlated with neuronal injury and irreversible brain damage (Tanaka et al., 1996). In animal models, signs of neuropathology are evident after 20 min of seizures, and seizures lasting 40 min are more difficult to terminate (Myhrer, 2007). Thus, understanding how seizures are initiated or inhibited at the cellular level is critical for understanding damage prevention and countermeasure development.

To understand the mechanism of seizures, previous studies (Spradling et al., 2011a, 2011b) have analyzed the transcriptomic responses in five nerve agent-sensitive brain regions (Aroniadou-Anderjaska et al., 2009; Myhrer, 2007; Myhrer et al., 2007) at different time intervals (0.25 h, 1 h, 3 h, 6 h, and 24 h) in rats following sarin-induced seizures. In these experiments, approximately half of the rats exposed to a $1 \times LD_{50}$ (median lethal dose) concentration of sarin developed seizures (Fig. 1a). The extensive activation of inflammatory responses in the seizing rats, in particular by neurotoxic and pro-inflammatory cytokines, such as Il-1β, Tnf-α, and Il-6, was observed in all brain regions (Spradling et al., 2011a, 2011b), consistent with findings from previous studies (Chapman et al., 2006; Dhote et al., 2007; Dillman et al., 2009; Johnson and Kan, 2010; Svensson et al., 2001, 2005; Williams et al., 2003).

In contrast, our study was focused on the roughly half of the sarin-exposed rats that did not develop seizures (Spradling et al., 2011a). Even though half of the animals given a dose of $1 \times LD_{50}$ are affected by seizures leading to death (if left untreated), the remaining non-seizing animals will be affected by sarin at these exposure levels, and non-lethal damage and subsequent long-term sequelae cannot be ruled out for this group. The differences in seizure onset are part of the natural variability in the biological response capacity to overcome the sarin insult and do not represent an experimental artifact. Thus, this study addresses the difference in this biological response and attempts to identify the underlying molecular pathways responsible for avoiding the onset of seizures in the non-seizing group.

We hypothesized that endogenous neuroprotective mechanisms helped this group respond to sarin intoxication and that this response was reflected in global changes in RNA levels from non-seizing rats. Thus, a comparison between seizing and non-seizing sarin-exposed rats could help us better understand both pathological and defense mechanisms involved in the response to OP nerve agents. These mechanisms could further be exploited for implementing novel potential neuroprotective therapeutic strategies that could mediate the cascade of secondary events leading to brain damage (Tang et al., 2011). Importantly, our analysis determined that there were both commonalities and substantial differences between the seizing and non-seizing rat responses, and that the non-seizing response was specifically tied to neuroprotection via the differential activation of anti-inflammatory and anti-apoptotic pathways.

2. Results

We determined the differentially expressed genes (DEGs) from the data of Spradling et al. (2011b), using a false discovery rate (FDR)-corrected *p*-value cutoff of 0.05 for each of the five studied brain regions (amygdala, hippocampus, piriform cortex, septum, and thalamus) and at each time point (0.25 h, 1 h, 3 h, 6 h, and 24 h). The determination of the DEGs from each cohort of seizing and non-seizing rats employed separate control groups due to differences in countermeasure administration. Seizing rats and their controls were given the countermeasures atropine sulfate and 2-pyridine aldoxime methylchloride (2-PAM) within one minute of seizure onset, followed by the anticonvulsant diazepam 0.50 h later. Non-seizing rats and their controls received no countermeasures (Fig. 1a). Fig. 1b and c shows the total number of up- and down-regulated genes in either group as a function of time and brain region. Although the number of DEGs was comparable at 0.25 h, the continued transcriptional response in the seizing group was substantial and still increasing at 24 h. In