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Chemical Profile

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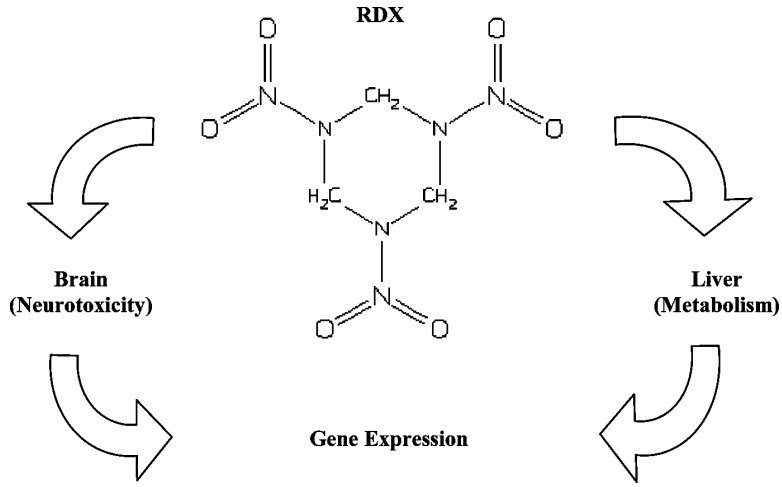

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Global Gene Expression in Rat Brain and Liver after Oral Exposure to the Explosive Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

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RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) is a synthetic, high-impact, relatively stable explosive that has been in use since WWII. Exposure to RDX can occur in occupational settings (e.g., during manufacture) or through the inadvertent ingestion of contaminated environmental media such as groundwater. The toxicology of RDX is dominated by acute clonic-tonic seizures at high doses, which remit when exposure is removed and internal RDX levels decrease. Subchronic studies have revealed few other measurable toxic effects. The objective of this study was to examine the acute effects of RDX on the mammalian brain and liver using global gene expression analysis based on a predetermined maximum internal dose. Male Sprague−Dawley rats were given a single, oral, nonseizure-inducing dose of either 3 or 18 mg/kg RDX in a gel capsule. Effects on gene expression in the cerebral cortex and liver were assessed using Affymetrix Rat Genome 230 2.0 whole genome arrays at 0, 3.5, 24, and 48 h postexposure. RDX blood and brain tissue concentrations rapidly increased between 0 and 3.5 h, followed by decreases at 24 h to below the detection limit at 48 h. Pairwise comparison of high and low doses at each time point showed dramatic differential changes in gene expression at 3.5 h, the time of peak RDX in brain and blood. Using Gene Ontology, biological processes that affected neurotransmission were shown to be primarily down-regulated in the brain, the target organ of toxicity, while those that affected metabolism were up-regulated in the liver, the site of metabolism. Overall, these results demonstrate that a single oral dose of RDX is quickly absorbed and transported into the brain where processes related to neurotransmission are negatively affected, consistent with a potential excitotoxic response, whereas in the liver there was a positive effect on biological processes potentially associated with RDX metabolism.

Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine is a synthetic, rapid detonation explosive (RDX), royal demolition explosive, hexogen, and cyclonite and has been used in munitions and formulations since World War II. RDX is an N-nitrated, six member heterocyclic ring compound (see Figure 3), with an empirical formula $C_3H_6N_6O_6$ and a molecular weight of 222.26 and is poorly soluble in water but soluble in organic solvents such as acetone or dimethyl sulfoxide (DMSO) (*1*). RDX has been found at 16 of the 1397 hazardous waste sites on the National Priorities List (*1*).

In mammals, high oral doses of RDX can cause rapid onset of seizures, which are transient; the speed of onset after a single high dose indicates a direct effect rather than that of its metabolite (*2, 3*). In humans, seizures have occurred in manufacturing where poor industrial hygiene practices were used, although such cases were significantly reduced with improved engineering controls. Recently, workers at a munitions plant in Turkey exhibited frank seizures due to RDX exposure but were released from the hospital after a few days without further adverse effects (*4*). Thus, there appear to be no long-term sequelae due to acute exposure-induced sporadic seizures, although there is at least one report to support neurobehavioral effects in long-term exposures (*5*).

The acute central nervous system effects found in humans have also been demonstrated in rodents, and a threshold plasma RDX level for seizures has been established. The reported LD_{50} for rodents ranges from 71 to 118 mg/kg for a single dose (*1*). No observable long-term effects or histopathologies have been reported in brains of rats that sporadically seized during a 90 day study (*6*) or in primates (*7*). Remarkably, in almost all animal studies of RDX, no effort has been made to characterize the acute neurotoxicity at a biochemical or molecular level, although some work has linked internal doses to seizures (*3*). Instead, studies have concentrated on the measurement of internal dose accompanied by organ histopathology. Thus, data continue to accumulate on the toxicokinetics (metabolism and distribution) of RDX, leaving that of toxicodynamics (interaction with molecular targets) lagging behind.

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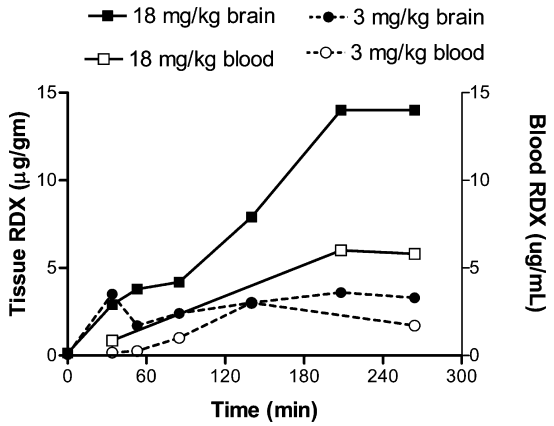

Figure 1. Rats were orally dosed with nominal doses of either 18 or 3 mg/kg RDX in a gel capsule, after which animals were euthanized at the various time points (see Table 1). Blood and brain RDX was measured for each dose and time point using gas chromatograph−electron capture detection.

Recently, some work has been carried out on the gene expression of RDX-exposed mammalian livers (*8*) in which it was reported that gene expression increased in biological pathways related to signaling, ubiquitin-dependent protein catabolism, carboxylic acid and lipid metabolism, and glutathione−conjugation reactions, suggesting active metabolism of RDX in liver. Here, we examined the effect of RDX in rodent brain and liver at 3.5, 24, and 48 h using analytical chemistry and gene expression arrays. These results represent the first published experiments examining the effects of RDX on gene expression in mammalian cerebral cortex.

Experimental Procedures

Chemicals. RDX was characterized using gas chromatography fitted with an electron capture detector (GC-ECD, see below) and was found to be ≥98% pure with the remainder being water. RDX was handled according to in-house protocols for hazardous materials/explosives. Dosing capsules were filled by the Directorate of Laboratory Sciences at the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) by weighing pure RDX into size 9 gel capsules (Torpac, Fairfield, NJ). Capsules were then individually stored in dark glass sample bottles for up to 2 days in a refrigerator until required for dosing.

Animals. Male Sprague−Dawley rats (Charles River Laboratories, Wilmington, MA) were pair-housed and had a 1 week acclimatization period and a 12/12 light/dark cycle, and food and water were provided ad libitum. At the time of dosing, animals weighing 300−400 g were administered a one time oral dose, either 3 or 18 mg/kg, of pure RDX in a gel capsule (Torpac) according to the manufacturer's instructions. A capsule dispenser was used to place the capsule in the stomach. Doses were calculated based on previous work, so as not to induce seizure, and animals were observed over the first hour to confirm lack of seizure. In a range-finding preliminary experiment at these doses, the time of maximum concentration of RDX in blood and brain was estimated to be 3.5 h (Figure 1), which was the earliest time point chosen for the microarray study. The mean and standard deviation of the actual dosage for 12 animals for each of the high and low doses were 5.09 ± 0.196 and 1.04 ± 0.574 mg, respectively. At times 0, 3.5, 24, and 48 h after dosing for the main study, animals ($n = 3$) were euthanized using decapitation, and the cerebral cortex was removed from the brain and immediately flash frozen in liquid nitrogen for the microarray analysis. The remainder of the brain was frozen for RDX analysis. Blood samples were placed into tubes with EDTA as an anticoagulant for RDX analysis. A segment of the median lobe of the liver was immediately frozen in liquid nitrogen for gene expression analysis, and samples were stored at −80 °C until RNA extraction. The remainder of the brain and liver was stored at −40 °C until analysis for RDX tissue concentrations could be conducted.

Table 1. Number of Significant Genes by Pairwise Comparison of High and Low Doses

	significant genes			
time (h)	cortex		liver	
0	1 (54)[a]	1[b] ▲	0 (917)	
3.5	2094 (2303)	721[b] ▲ 1373 ▼	911 (1224)	616 ▲ 295 ▼
24	0 (93)		0 (292)	
48	315 (1099)	76 ▲ 239 ▼	0 (343)	

[a] Significant genes from pairwise comparison of high and low doses before correction using Benjamini and Hochberg are in parentheses; the number outside the parentheses is after correction. [b] Genes found to be significant after background correction were further subdivided into up- or down-regulated genes.

The animal protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee, and the work was carried out in an AAALAC accredited facility. The study was carried out in accordance with the standards found in Title 40 Code of Federal Regulations, Part 792, Good Laboratory Practices (www.nal.usda.gov/awic/legislat/40cfr97.htm). The investigators and technicians adhered to the following guidelines: the *Public Health Service Policy on Humane Care and Use of Laboratory Animals*, "U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training", and the Animal Welfare Act (www.nap.edu/readingroom/books/labrats/).

Analytical Methods. Samples were analyzed using GC-ECD based on a previously published method (*9*). For whole blood samples, lysing of erythrocytes was carried out by adding 100 μL of mixed whole blood into 100 mL of deionized (DI) water in a volumetric flask. To extract RDX, 5 mL of isoamyl acetate (Pesticide Residue grade, anhydrous, Sigma-Aldrich, St. Louis, MO) was added to the 100 mL flask, followed by liquid/liquid extraction at room temperature for 1 h. Standards were made from pure RDX dissolved in acetonitrile (ACN) and spiked into DI water at 0, 0.01, 0.02, 0.1, 0.2, 0.5, 1.0, and 2.0 μg RDX/mL, which was extracted using the same procedure as for samples. An aliquot of the isoamyl acetate extract was then used for analysis. With this method, RDX and other organic molecules were extracted into the organic phase, while water soluble molecules were separated into the aqueous phase, significantly decreasing the extent of interference in the gas chromatogram that is seen with other methods for analysis of explosives.

Tissue samples were treated differently than blood samples. Flash frozen tissue was partially defrosted and blended in a tissue macerator with 20 mL of DI water to an even consistency. To this mix was added 5 mL of isoamyl acetate, followed by extraction in a flat-bed shaker for up to 3 h, depending on the tissue mass. The mixture was then centrifuged for 20 min to separate the isoamyl acetate extract from the tissue and water. An aliquot of the supernatant was then used for analysis.

As an additional quality control, a surrogate chemical, 3,4-dinitrotoluene (3,4-DNT), was added as an external running standard to each sample prior to extraction. DNT eluted just

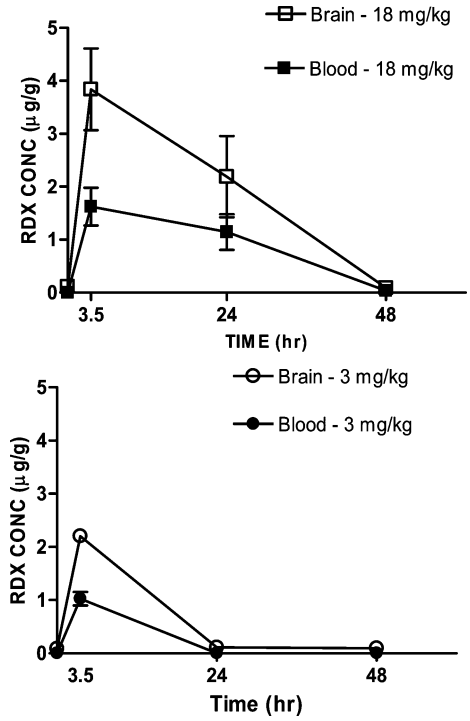

Figure 2. Main study. Uptake of RDX into blood and brain. Animals dosed with RDX were euthanized at the indicated time points, and after removal of the cerebral cortex, the remainder of the brain tissue was analyzed for RDX.

before RDX and was used to monitor the extraction efficiency, accuracy, and reproducibility for each sample. Blind controls were used for each sample extraction batch. In spiked samples of blood, brain, or liver, the reporting limits were approximately 0.10 μg/mL and 0.12 and 0.25 μg/g, respectively, with tissue recovery of spiked 3.4-DNT between 80 and 120%.

Microarray Analysis. All experiments were performed using the Affymetrix Rat Genome 230 2.0 whole genome oligonucleotide arrays (Santa Clara, CA). Frozen tissue was homogenized in Tri Reagent (Sigma Chemical Co., St. Louis, MO), and the RNA was extracted according to the manufacturer's protocol. RNA purification, microarray probe synthesis, hybridization, and microarray processing were as described previously (*10*).

Data Analysis. Data were imported into Gensifter (VizX Laboratories, Seattle, WA) (www.gensifter.net) and normalized using robust multiarray averaging (RMA) (*11*). For each time point, the average expression for each gene ($n = 3$) at the high dose was compared to the average expression at the low dose. This pairwise analysis (Student's t test) of high and low dose (at each time point) was carried out at $\alpha = 0.05$ and a 1.5-fold threshold to generate lists of differentially expressed genes. After a false discovery rate adjustment using Benjami and Hochberg (*12*), these lists were then gathered into classes based on gene ontology terms (biological processes), and each process was given a score based on the standardized difference score formula below (*13*):

$$z = \frac{\left(r - n\frac{R}{N}\right)}{\sqrt{n\left(\frac{R}{N}\right)\left(1 - \frac{R}{N}\right)\left(1 - \frac{n-1}{N-1}\right)}} \quad (1)$$

where r is the number of significant genes associated with a particular GO term (found genes), n is the number of genes that link to that GO term, N is the total number of genes measured, and R is the total number of genes from N meeting selection criteria. Absolute values of the Z scores greater than 1.96 are considered significant. Thus, the Z score measures the extent of overrepresentation or underrepresentation of a particular set of genes in a GO process.

The list of FDR-corrected significant genes was imported into an Excel file and filtered to genes greater than one, that is, at least two genes that affected a process. The lists of ontologies were then sorted based on the magnitude of the z value.

Results

A range-finding experiment (Figure 1) using both the high (18 mg/kg) and the low (3 mg/kg) doses showed that the RDX concentrations reached their maximum in both blood and brain approximately 3.5 h after dosing, using one animal per time point. In the main study, RDX in brain and blood peaked at 3.5 h (Figure 2) with a low or high mean concentration of 2.2 $\pm$ 0.1 or 3.8 $\pm$ 1.3 μg/g, respectively. While the internal concentrations due to the low dose fell below detection limits in both blood and brain by 24 h, the high dose persisted past 24 h but was undetectable by 48 h. The measured concentrations of RDX in liver samples were variable, with some samples

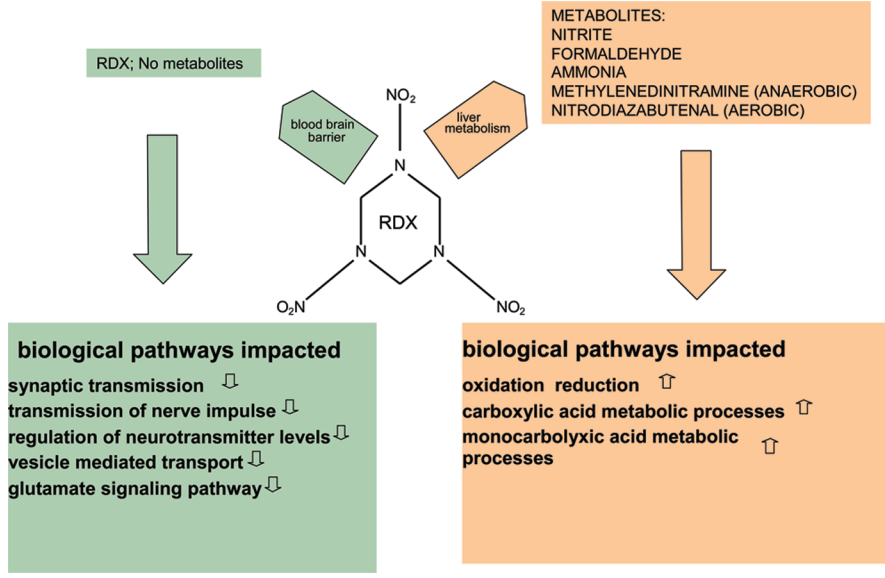

Figure 3. Schematic diagram of the acute effects of RDX in brain and liver, based on the findings in this study.

Table 2. Biological Processes Significantly Altered in the Cerebral Cortex

ontology: high vs low dose, time 3.5 h	list	up	down	array	*z*-up	*z*-down
synaptic transmission	54	6	48	298	−0.93	6.06
transmission of nerve impulse	62	8	54	354	−0.74	5.99
synaptic vesicle transport	13	1	12	38	−0.1	5.83
regulation of neurotransmitter levels	30	3	27	138	−0.51	5.67
vesicle-mediated transport	69	14	55	378	0.95	5.66
macromolecule localization	89	15	74	578	−0.46	5.41
glutamate signaling pathway	10	2	8	22	1.73	5.3
ameboidal cell migration	3	0	3	4	−0.35	5.25
protein transport	72	9	63	482	−1.4	5.14
establishment of protein localization	72	9	63	484	−1.41	5.1
protein localization	83	13	70	556	−0.82	5.09
negative regulation of hormone biosynthetic process	2	0	2	2	−0.24	5.08
negative regulation of hormone metabolic process	2	0	2	2	−0.24	5.08
transport	225	45	180	1830	−1.29	4.96
small GTPase mediated signal transduction	56	10	46	327	0.17	4.9
cell-cell signaling	70	11	59	463	−0.7	4.75
neurotransmitter secretion	21	2	19	98	−0.51	4.69
ionotropic glutamate receptor signaling pathway	6	1	5	12	1.12	4.62
ribonucleoprotein complex assembly	13	1	12	50	−0.38	4.61
establishment of localization	229	49	180	1873	−0.85	4.6
bleb formation	3	0	3	5	−0.39	4.57
regulation of translation	14	1	13	60	−0.57	4.35
synaptic vesicle exocytosis	8	1	7	23	0.41	4.32
cerebellar Purkinje cell layer development	4	0	4	9	−0.52	4.32
synaptic vesicle endocytosis	4	0	4	9	−0.52	4.32
localization	262	61	201	2190	−0.39	4.19
positive regulation of protein metabolic process	20	2	18	101	−0.56	4.15
axon cargo transport	5	0	5	14	−0.65	4.13
branched chain family amino acid catabolic process	3	0	3	6	−0.42	4.06
serotonin metabolic process	3	0	3	6	−0.42	4.06
Golgi vesicle budding	5	1	4	10	1.34	4.01
anterograde axon cargo transport	2	0	2	3	−0.3	3.98
microvillus biogenesis	2	0	2	3	−0.3	3.98
microvillus organization and biogenesis	2	0	2	3	−0.3	3.98
nuclear translocation of MAPK	2	0	2	3	−0.3	3.98
nucleotide transport	2	0	2	3	−0.3	3.98
purine nucleotide transport	2	0	2	3	−0.3	3.98
positive regulation of cellular protein metabolic process	19	2	17	97	−0.5	3.96
organelle fusion	7	1	6	20	0.56	3.95
establishment of localization in cell	93	21	72	654	0.49	3.93
regulation of cellular pH	5	0	5	15	−0.67	3.92
cell morphogenesis	62	15	47	386	1.18	3.87
cellular structure morphogenesis	62	15	47	386	1.18	3.87
cellular localization	97	23	74	681	0.77	3.87
posttranscriptional regulation of gene expression	19	3	16	92	0.21	3.8
cell differentiation in hindbrain	4	0	4	11	−0.57	3.74
cerebellar cortex formation	4	0	4	11	−0.57	3.74
initiation of viral infection	4	0	4	11	−0.57	3.74
vesicle organization and biogenesis	10	1	9	41	−0.18	3.66
cerebellar Purkinje cell differentiation	3	0	3	7	−0.46	3.65

showing little or no RDX, while others showed concentrations similar to the brain, possibly due to metabolism of RDX prior to measurement.

Table 1 shows the number of significant genes recovered from a pairwise statistical analysis of high vs low doses at each of the four time points. These numbers were adjusted for false discovery using Benjamini and Hochberg (numbers in parenthesis) and then further subdivided into up- or down-regulated genes (black arrows, Table 1). Overall, the most dramatic changes in gene expression took place at the 3.5 h time point, for both cerebral cortex and liver (Table 1). In cerebral cortex tissue, a total of 2094 genes were found to be changed in expression in the high dose relative to the low dose at 3.5 h, with two-thirds of these genes down-regulated (1373 genes). The opposite effect took place in the liver, where out of a total of 911 genes, two-thirds were up-regulated. When the total number of significant genes for cortex and liver was compared, only 71 genes were found to be common to both tissues, and a majority of these were unknown genes (listed as "transcribed locus"). Thus, the majority of differentially expressed genes was unique to either the brain or the liver, and overall gene expression was decreased in response to RDX in the brain and increased in the liver.

Significant genes (1.5-fold, $\alpha = 0.05$, FDR correction) were examined for biological meaning using GO, where a series of *Z* score results for each biological process were established. *Z* score tabulations are an aggregate score (see the Experimental Procedures) for the number of genes up- or down-regulated for a particular biological process. The magnitude and direction of the score (negative or positive) indicate whether the genes grouped in these processes are over- or under-represented on average, as compared to the total number of genes on the array for that biological process. Values greater or less than 2.0 are considered significant for a particular process. Biological processes most highly affected for brain and liver are listed in Tables 2 and 3, respectively.

For cerebral cortex, there was a remarkable under-representation of biological processes that related to neurotransmission (boldface, Table 2) when the high dose was compared to the low dose at a maximal brain concentration of RDX (3.5 h). In

Table 3. Biological Processes Significantly Altered in the Liver

ontology	list	up	down	array	*z*-up	*z*-down
oxidation reduction	38	34	4	311	6.49	−0.36
carboxylic acid catabolic process	8	8	0	33	6.02	−0.72
organic acid catabolic process	8	8	0	33	6.02	−0.72
fatty acid beta-oxidation	6	6	0	22	5.65	−0.59
pyrimidine base catabolic process	2	2	0	3	5.6	−0.22
regulation of cytolysis	2	2	0	3	5.6	−0.22
thymine catabolic process	2	2	0	3	5.6	−0.22
thymine metabolic process	2	2	0	3	5.6	−0.22
fatty acid catabolic process	7	7	0	31	5.36	−0.7
acetyl-CoA catabolic process	5	5	0	18	5.22	−0.53
fever	3	3	0	8	4.89	−0.35
germ cell migration	3	3	0	8	4.89	−0.35
positive regulation of Actin filament polymerization	3	3	0	8	4.89	−0.35
coenzyme catabolic process	5	5	0	20	4.86	−0.56
aspartyl-tRNA aminoacylation	2	2	0	4	4.75	−0.25
nucleobase catabolic process	2	2	0	4	4.75	−0.25
pinocytosis	2	2	0	4	4.75	−0.25
regulation of fatty acid beta-oxidation	2	2	0	4	4.75	−0.25
synaptic growth at neuromuscular junction	2	2	0	4	4.75	−0.25
lamellipodium biogenesis	4	4	0	15	4.54	−0.48
acetyl-CoA metabolic process	6	6	0	31	4.43	−0.7
cofactor catabolic process	5	5	0	23	4.41	−0.6
cellular catabolic process	47	36	11	475	4.23	1.43
cellular lipid metabolic process	45	35	10	460	4.19	1.15
tricarboxylic acid cycle	4	4	0	17	4.17	−0.51
body morphogenesis	2	2	0	5	4.15	−0.28
carnitine metabolic process	2	2	0	5	4.15	−0.28
cellular lipid catabolic process	10	9	1	65	4.14	0
catabolic process	50	39	11	549	3.97	0.93
lipid metabolic process	46	36	10	507	3.8	0.83
heat generation	3	3	0	12	3.76	−0.43
apical protein localization	2	2	0	6	3.71	−0.31
nuclear membrane organization and biogenesis	2	2	0	6	3.71	−0.31
nucleobase biosynthetic process	2	2	0	6	3.71	−0.31
pyrimidine base metabolic process	2	2	0	6	3.71	−0.31
transcription from RNA polymerase III promoter	2	2	0	6	3.71	−0.31
aerobic respiration	4	4	0	20	3.71	−0.56
lipoprotein biosynthetic process	6	6	0	39	3.69	−0.78
monocarboxylic acid metabolic process	21	19	2	221	3.63	−0.77
lipid modification	8	8	0	63	3.6	−0.99
cellular iron ion homeostasis	5	4	1	21	3.57	1.21
carboxylic acid metabolic process	40	30	10	415	3.56	1.49
organic acid metabolic process	40	30	10	416	3.54	1.49
fatty acid oxidation	6	6	0	42	3.47	−0.81
lipid oxidation	6	6	0	42	3.47	−0.81
synaptogenesis	5	4	1	22	3.45	1.15
diterpenoid metabolic process	3	3	0	14	3.38	−0.47
nucleobase metabolic process	3	3	0	14	3.38	−0.47
positive regulation of protein polymerization	3	3	0	14	3.38	−0.47
retinoid metabolic process	3	3	0	14	3.38	−0.47

fact, the top five processes negatively impacted were related to neurotransmission, including synaptic transmission and vesicle transport. The glutamate receptor signaling pathway was also down-regulated. This downturn in mRNA expression was also evident at the 48 h period for a similar set of biological processes associated with neurotransmission, although the effect was not as widespread or as large in magnitude (data not shown).

For liver tissue (Table 3), the effect on biological processes was not as dramatic as that of cortex. Oxidation reduction was over-represented in the high dose, as were carboxylic acid catabolic processes. Metabolic processes were the most affected, as would be expected when there is a rapid and acute inflow of xenobiotic into hepatocytes. The highest *Z* scores were for oxidation reduction and carboxylic acid metabolism.

Discussion

The maximal internal dose of RDX is a critical determinant in the acute neurological effects observed on laboratory animals (*2*) and was used here to design a microarray expression analysis study. For blood and brain, the peak internal dose (without observed seizure) occurred at about 3.5 h. After 48 h, RDX in tissues decreased to baseline, and both doses were assumed to be eliminated (below the method detection limit). Blood and brain concentrations of RDX tightly paralleled each other (Figure 2) on both sides of the peak at 3.5 h, indicating that RDX readily moves between blood and tissues but does not accumulate (since the brain did not lag behind blood) and is eliminated quickly from the brain at this dose.

Differential gene expression was explored by comparing the high and low dose at each time point. The dramatic differential expression at the time of peak absorption of RDX into the brain (3.5 h) demonstrated an acute and rapid response of mRNA to RDX. Significantly, this change was predominantly downward for processes related to the generation, packaging, mobilization, and release of neurotransmitters, as well as other potentially related processes. Apoptosis (or necrosis) was not identified as a significantly affected process in ontological analysis, and it can therefore assume that the observed down-regulation at high doses was not an experimental bias due to neuronal injury or death. Significantly, the glutamate signaling pathway was down-

regulated in the high dose, indicating a potential mechanism of action of RDX via excessive glutamate stimulation. The decrease in expression of these processes could be a neurological response to excitotoxicity, in that the machinery that makes, packages, and activates neurotransmitters may be depressed as a negative response to the increase in seizure likelihood from RDX influx into the brain.

RDX metabolism is thought to be initiated by P450 or P450-like enzymes by which the nitroso groups are cleaved, releasing nitrite molecules, followed by the hydroxylation of the carbon atoms. Nitrite and formaldehyde are produced, and one of two intermediate metabolites, 4-nitro-2,4-diazabutanal (NDAB) or methelyenedinitramine (MEDINA) (*14*), under aerobic or anaerobic conditions, respectively (Figure 3). Consistent with this model, oxidation−reduction processes were overrepresented. These results are similar to those found in livers of rats orally dosed with RDX and examined 24 h after exposure (*8*) where expression was increased in pathways related to signaling, ubiquitin-dependent protein catabolism, carboxylic acid and lipid metabolism, and glutathione−conjugation reactions.

This work showed that a high, but nonseizure-inducing, dose of RDX caused down-regulation of two-thirds of the significantly changed genes in cerebral cortex when compared to a lower dose and that many of these genes were related to specific biological processes governing neurotransmission, suggesting a hypothesis of glutamate excitoxicity. Liver, on the other hand, had two-thirds of the significant genes up-regulated in response to the higher RDX dose, and some of these processes were related to the metabolism of nitrogen compounds, which are contained in the RDX molecule. A recent publication examined the effect of low doses of RDX in mice on the expression of miRNA species in brain and liver (*15*), but the calculated downstream targets of miRNA were not verified, making comparison with this work difficult.

Overall, this study showed that there are distinct effects by RDX on the cerebral cortex and liver and that the target organ of toxicity, the brain, responds to high RDX levels by down-regulation of important processes associated with neurotransmission.

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