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The mRNA expression and histological integrity in rat forebrain motor and sensory regions are minimally affected by acrylamide exposure through drinking water

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ABSTRACT

A study was undertaken to determine whether alterations in the gene expression or overt histological signs of neurotoxicity in selected regions of the forebrain might occur from acrylamide exposure via drinking water. Gene expression at the mRNA level was evaluated by cDNA array and/or RT-PCR analysis in the striatum, substantia nigra and parietal cortex of rat after a 2-week acrylamide exposure. The highest dose tested (maximally tolerated) of approximately 44 mg/kg/day resulted in a significant decreased body weight, sluggishness, and locomotor activity reduction. These physiological effects were not accompanied by prominent changes in gene expression in the forebrain. All the expression changes seen in the 1200 genes that were evaluated in the three brain regions were ≤1.5-fold, and most not significant. Very few, if any, statistically significant changes were seen in mRNA levels of the more than 50 genes directly related to the cholinergic, noradrenergic, GABAergic or glutamatergic neurotransmitter systems in the striatum, substantia nigra or parietal cortex. All the expression changes observed in genes related to dopaminergic function were less than 1.5-fold and not statistically significant and the 5HT1b receptor was the only serotonin-related gene affected. Therefore, gene expression changes were few and modest in basal ganglia and sensory cortex at a time when the behavioral manifestations of acrylamide toxicity had become prominent. No histological evidence of axonal, dendritic or neuronal cell body damage was found in the forebrain due to the acrylamide exposure. As well, microglial activation was not present. These findings are consistent with the absence of expression changes in genes related to changes in neuroinflammation or neurotoxicity. Over all, these data suggest that oral ingestion of acrylamide in drinking water or food, even at maximally tolerable levels, induced neither marked changes in gene expression nor neurotoxicity in the motor and somatosensory areas of the central nervous system.

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Introduction

Movement deficits and impairment of somatosensory perception have been reported in both humans and laboratory animals after either acute or chronic exposure to acrylamide (Spencer and Schaumburg, 1974a, 1974b; Sickles et al., 1996, 2002, 2007). This is known to be in part due to damage to neuronal processes residing in the peripheral somatosensory, lower motor neuron, spinal chord and brain stem regions. Most of the acrylamide-induced neurologically related deficits appear to be reversible with time in both humans and laboratory animals (Crofton et al., 1996; Hagmar et al., 2001). As well, neuronal damage/impairment in areas of the central nervous system (CNS) in rat forebrain have been reported after treatment with acrylamide (Lehning et al., 2003).

The studies reported here were designed as a mechanistic adjunct to a comprehensive evaluation of acrylamide neurotoxicity from lifetime exposure in male and female Fischer 344 rats including fetal and neonatal exposures, which are nearing completion at this institution. The goals for the latter study are to critically evaluate dose–response for AA exposures (approximately 0.1, 0.2, 0.6, 3 mg/kg bw/d consumed in drinking water) that are closer to a typical human dietary exposure from cooked foods (≤1 μg/kg bw/d) and to provide a more precise estimate of human neurotoxic risks. Although the rat strain and oral route of exposure are the same, the maximum acrylamide dose chosen for the mechanistic study is necessarily higher (44 mg/kg bw/d, which

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approaches that maximally tolerable) than those for the lifetime exposure because of the large time differential (50-fold).

This 14 day exposure time (defined as a pre-chronic type by NTP) permitted a determination whether subchronic exposures of rats to high levels of acrylamide altered gene expression in the forebrain and the neuroendocrine system. In addition, potential damage to the forebrain from acrylamide exposure was evaluated histologically. We have previously reported the results of this subchronic acrylamide exposure on the hypothalamus, pituitary and thyroid function for the neuroendocrine system (Bowyer et al., 2008). We now report the gene expression profiles in three brain regions involved in locomotor movement and somatosensory perception to determine whether they had been altered by the subchronic acrylamide exposure. We chose a 14-day acrylamide exposure in male F344 rats, and tested the effects of three different levels of exposure to acrylamide, which was administered via drinking water. The highest exposure tested was equivalent to approximately 50 mg/kg/day acrylamide. At this high dose, cumulative exposure is in the range of previous studies that produced pronounced movement deficits, decreased locomotor activity and significant body weight reductions by other investigators (Crofton et al., 1996; Barber et al., 2001a); Beland, F.A., personal communication).

The striatum, substantia nigra and parietal cortex are very important forebrain regions involved in locomotor movement and somatosensory perception (parietal cortex) (Gerfen, 1984; Carlsson, 1987; Graybiel, 1990; Zilles and Wree, 1995; Hornykiewicz, 2001; Jones, 2002a, 2002b; Baker, 2007). In addition, data were obtained from several histological methods to evaluate the integrity of the brain, and to look for evidence of neurodegeneration and neuroinflammation. It was anticipated that alterations in gene expression in these regions might possibly occur indirectly based on the previously documented acrylamide-related damage occurring in neurons in the spinal chord and periphery, as well as putative effects of acrylamide on neurotransmission (Spencer and Schaumburg, 1974a, 1974b; Crofton et al., 1996; Sickles et al., 1996, 2002, 2007; Hagmar et al., 2001; Lehning et al., 2003b). It was also anticipated that changes in forebrain histology might occur with the acrylamide exposure, which would be expected to increase the likelihood of significant changes in gene expression in striatum, substantia nigra and parietal cortex.

Particular interest was placed on genes related to the dopaminergic as well as serotonergic, cholinergic, GABAergic and glutamatergic neurotransmitter systems in the striatum or substantia nigra regions of the basal ganglia since they play such an important role in controlling locomotor movement (Carlsson, 1987; Hornykiewicz, 2001; Galvan and Wichmann, 2007; Mora et al., 2008; Morelli et al., 2007; Pisani et al., 2007; Quik et al., 2007; Schiffmann et al., 2007; Tepper and Lee, 2007). We focused on gene expression changes in the basal ganglia because this is a region that controls locomotor movement and when damaged by Parkinson's and Huntington's disease causes severe movement deficits (Hornykiewicz, 2001; Fleming and Chesselet, 2006; Galvan and Wichmann, 2007; Lobsiger and Cleveland, 2007; Ramaswamy et al., 2007). As well, basal ganglia regions have been reported to be sensitive to neurotoxic insults due to exposure to various compounds and metals including; manganese, paraquat, rotenone, 3-nitropropionic acid, MPTP and amphetamines (Langston et al., 1983; Ludolph et al., 1991; Greenamyre et al., 2003; Barlow et al., 2007; Fleckenstein et al., 2007; Perl and Olanow, 2007).

Methods

Acrylamide exposure. Procedures involving the use of rats were reviewed and approved by the NCTR Laboratory Animal Care and Use Committee, and the studies were carried out in accordance with the declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. All the male F344 rats used in this study were obtained from the NCTR colony. They were maintained on the basal diet (Purina 5LG6 meal containing <50 ppb acrylamide; Twaddle et al., 2004) from weaning on postnatal day 21 until approximately 70 days of age and housed two per cage. The acrylamide (>99.9% purity, CAS 79-06-1) used for dosing was purchased from Sigma Chemical Co. (St. Louis, MO). Acrylamide was administered in the drinking water at concentrations of 25, 100, and 500 μg/ml, concentrations chosen to administer daily doses of approximately 2.5, 10, and 50 mg/kg bw/d. Concentrations of acrylamide dosing solutions were changed weekly and measured as previously reported (Bowyer et al., 2008). Based on twice weekly measurements of cage-average body weights and drinking water consumption, the average doses delivered were 85–88% of the estimated 50 mg/kg dose. Thus the actual cumulative dose was between 43 and 44 mg/kg/day. The 500 μg/ml in drinking water exposure was deemed to be the maximal tolerable, over a 2 week period, because 1000 μg/ml was found to be lethal within 1 week in preliminary experiments. While blood levels of acrylamide were not directly measured in the present experiments, this exposure regimen is predicted from a validated PBPK model to yield 'steady state' serum concentrations of approximately 30 μM and 20 nmol/g in brain during waking hours when water is being consumed. Rats had access to the drinking water containing acrylamide continuously until removal at termination.

Termination and tissue harvest for cDNA array data collection. After exposure, the rats were terminated for the harvesting of tissues between 8:00 and 10:00 AM and the order of termination was rotated between dose groups to minimize the unintended effects of circadian rhythm. Rats were terminated by decapitation, and their brains were rapidly removed and chilled in 4 °C normal saline. The hypothalamus, substantia nigra, striatum, and parietal cortex were saved for analysis. All tissues were dissected bilaterally on ice, immediately frozen on dry ice, and then transferred to −70 °C storage. The dissections of the midbrain region containing substantia nigra (−4.4 anterior to −6.4 posterior, Paxinos and Watson, 1995) and entire striatum (45–55 mg) were performed in the same manner as that previously reported (Bowyer et al., 2007). The area of the parietal cortex dissected was slightly greater than previously used (0.0 anterior to −3.5 posterior) to include most of the hindlimb and trunk somatosensory region.

RNA isolation and cDNA array hybridization and imaging. cDNA array analysis and RT-PCR analysis was used to determine acrylamide's effects on gene expression after isolation of total RNA from various brain regions. Methods similar to those previously described (Freeman et al., 2000; Bowyer et al., 2008) were used to isolate total RNA from brain tissue and hybridize the ^{32}P-cDNA generated from this total RNA with cDNA macroarray screens. Total cellular RNA was isolated using Tri Reagent (Molecular Research Center Inc., Cincinnati, OH) (Chomczynski and Mackey, 1995). RNA concentrations ranged from 2.5 to 3.5 μg/μl (quantified using the Agilent 2100 Bioanalyzer using the RNA 6000 Nano Assay; Agilent Tech., Palo Alto, CA), and were stored at −70 °C. The evaluation of gene expression used Atlas 1.2K rat array types I (cat # 7854) via the procedures suggested by manufacturer (Clontech; Palo Alto, CA) with slight modifications (Bowyer et al., 2008). The hybridized arrays were exposed on phosphor image screens for 1 to 3 days and the phosphorescent images were then read using a Storm 860 PhosphorImager (Molecular Dynamics), and target signals were quantified using the AtlasImage 2.01 software provided by Clontech (Palo Alto, CA).

Statistical analysis. The array data were generated using a total of 44 individual high quality arrays. For the striatum there was an equal $n=7$ for both controls and the high dose acrylamide group and each array used was generated from a different animal in the exposure group. Likewise, an equal $n=7$ for control and the acrylamide group was used for substantia nigra region array analysis, and equal $n=8$ for control and the acrylamide group for parietal cortex analysis. Samples from each treatment were paired and processed concurrently