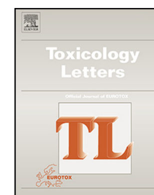
Toxicology Letters 236 (2015) 60–68

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet



Acrylamide increases dopamine levels by affecting dopamine transport and metabolism related genes in the striatal dopaminergic system

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HIGHLIGHTS

- The effect of oral acrylamide on neurobehavioral function in rats was assessed.
- ACR exposure resulted in increased striatal dopamine with a decrease in its metabolites.
- These changes correlated to genes modulating dopamine metabolism and transport.

ARTICLE INFO

Article history:
Received 4 December 2014
Received in revised form 28 April 2015
Accepted 30 April 2015
Available online 2 May 2015

Keywords:
Acrylamide
Dopamine
TH
MAO
DAT
VMAT2

ABSTRACT

Dopaminergic system dysfunction is proved to be a possible mechanism in acrylamide (ACR) -induced neurotoxicity. The neurotransmitter dopamine (DA) has an increasingly important role in the dopaminergic system. Thus, the goal of this study is to evaluate effects of ACR on dopamine and its metabolite levels, dopamine transport and metabolic gene expression in dopaminergic neurons. Male Sprague–Dawley (SD) rats were dosed orally with ACR at 0 (saline), 20, 30, and 40 mg/kg/day for 20 days. Splayed hind limbs, reduced tail flick time and abnormal gait which preceded other neurologic parameters were observed in the above rats. ACR significantly increased dopamine levels, decreased 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) contents in an area dependent manner in rat striatum. Immunohistochemical staining of the striatum revealed that the number of tyrosine hydroxylase (TH) positive cells significantly increased, while monoamine oxidase (MAO) positive cells were drastically reduced, which was consistent with changes in their mRNA and protein expressions. In addition, dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) expression levels were both down-regulated in the striatum. These results suggest that dopamine levels increase significantly in response to ACR, presumably due to changes in the dopamine transport and metabolism related genes expression in the striatal dopaminergic neurons.

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1. Introduction

There has been widespread concern about acrylamide (ACR) in many cooked starchy foods because of its possible neurotoxicity in humans and animals (Das and Srivastav, 2012; Foot et al., 2007; Lineback et al., 2012; Pruser and Flynn, 2011; Sharp, 2003). Human exposure to ACR is also generally believed to occur in workplaces or other environments, including laboratories and households. Indeed, sub-chronic low level work exposure to ACR may cause ataxia, gait changes, skeletal muscle weakness, skin abnormalities, and numbness of the hands and feet. In experimental animals

Abbreviations: ACR, acrylamide; DA, dopamine; DOPAC, 3, 4-dihydroxyphenylacetic acid; HVA, homovanilic acid; DAT, dopamine transporter; VMAT2, vesicular monoamine transporter 2; TH, tyrosine hydroxylase; MAO, monoamine oxidase; HPLC, high-performance liquid chromatographic; RT-PCR, reverse transcription polymerase chain reaction.

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http://dx.doi.org/10.1016/j.toxlet.2015.04.017
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(such as rodents, Guinea pigs, rabbits, cats and dogs) repeated daily exposure to this chemical (0.5–50 mg/kg/day) leads to neurological signs resembling the kind of neurotoxicity observed in humans (Edwards and Parker, 1977). In 1994, the international agency for research on cancer classified ACR as potentially carcinogenic to humans. However, the underlying mechanism of ACR-induced neurotoxicity is still poorly understood. It is classified as a neurotoxin and there are three important hypotheses considering ACR neurotoxicity: inhibition of kinesin-based fast axonal transport, alteration of neurotransmitter levels, and direct inhibition of neurotransmission (Erkekoglu and Baydar, 2014). Only a limited number of studies have been done to evaluate the effects of ACR on dopaminergic neurons in vivo.

Dopamine (DA) is a critical neurotransmitter under physiological conditions, and mainly distributes in the striatum. Striatum which is identified as the largest integrated processing element in the basal ganglia contains a large number of dopaminergic neurons (Li et al., 2011). DA in the neural pathways of dopaminergic system is synthesized, transported, released, and degraded on the presynaptic membrane. Previous studies have shown that ACR-induced neurotoxicity involves covalent binding of ACR to critical pre-synaptic protein thiol groups, which leads to a decrease in neurotransmitter release. In addition, it has been reported that acrylamide affects DA receptor density, DA uptake and ^{3}H -dopamine release (Hunt and Dalton, 1981). However, there is further evidence that demonstrates that DA may also serve as a neurotoxin participating in the neurodegenerative process, including ischemia and hypoxia after expose to high concentrations of stimulating amino acids (Buisson et al., 1992; Filloux and Wamsley, 1991). The DA availability in animal striatum significantly increases in ischemia. In the case of hypoxia, the striatal DA level rises by 1100%, compared to the control level (Akiyama et al., 1991). Similarly, once the DA balance is disrupted, the body would suffer impairments on motor coordination and balance impairment, learning and memory skills impediment. These neurological signs and symptoms are primarily caused by changes in DA level, which results from the selective loss of dopaminergic neurons in substantia nigra. The ratio (DOPAC + HVA)/DA is considered to be an appropriate index of DA turnover (with a higher ratio indicating higher turnover), reflecting the DA metabolism (Thiffault et al., 2000).

Functionally important genes in nerve cells and terminals play a critical role in maintaining the DA balance in the cytoplasm and the synaptic cleft (Blackburn et al., 1992). Among these genes, the dopamine transporter (DAT) is a vital factor for accumulation and compartmentalization of DA (Chotibut et al., 2012; Jaber et al., 1999; Jones et al., 1998). These transporters work at the plasma and vesicular membranes of dopaminergic neurons, respectively, and regulate DA levels in neuronal compartments. In heterozygous DAT KO mice, altered DAT expression affects age-related changes in dopaminergic function by transport of cytosolic DA (Hall et al., 2014). Vesicular monoamine transporter type 2 (VMAT2) takes cytosolic monoamines from intracellular vesicles, and discharges them into the extracellular space by exocytosis (Erickson et al., 1992). Therefore, VMAT2 dysfunction causes accumulation of free DA in cytoplasm, and finally leads to dopaminergic neuronal death (Brighina et al., 2013; Sala et al., 2010). Some surveys show that ACR inhibits DA uptake of synaptic vesicle in rat striatum, and alters DA levels in neurons (Barber et al., 2007). Likewise, the intracellular levels of DA partly depend on the activity of DA synthesizing and degrading enzymes. Tyrosine hydroxylase (TH) is a rate-limiting DA synthesis enzyme (Kaushik et al., 2007), and monoamine oxidase (MAO) represents one of the major metabolic enzymes for biogenic amine degradation (Pizzinat et al., 2003). Homovanilic acid (HVA) and 3, 4-dihydroxyphenylacetic acid (DOPAC) are two primary metabolites of DA metabolism via MAO. The TH positive cells usually are recognized as dopaminergic neurons. Parkinson's disease (PD) is associated with neurodegeneration of the nigrostriatal tract and loss of TH and DA (Khan et al., 2012). However, the studies on the effect of ACR on the striatal dopaminergic system in vivo have been quite limited. Hence, it is crucial to investigate the potential mechanism of ACR neurotoxicity through measuring DA levels and by studying effects of ACR on expression of functional proteins related to DA synthesis, transport and degradation in vivo.

2. Material and methods

2.1. Reagents

Acrylamide (ACR), dopamine (DA) and its metabolites: 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanilic acid (HVA) standards and other chemicals (all 99.9% purity) were obtained from Sigma (USA). All solvents were of analytical grade and Milli-Q water was used throughout the analyses. Hypersil ODS2 chromatographic column (4.6 mm × 250 mm, 5 μm) was purchased from Elite Analytical Instruments Company (Dalian, China). Trizol for RNA extraction was acquired from Invitrogen (USA). All primers for PCR were provided by Biological Engineering Technology Company (Shanghai, China). First strand cDNA Synthesis Kits and SYBR Green Quantitative kits for Real time-PCR were purchased from Fermentas Company (USA). RIPA, PMSF and BCA protein quantification kits were sourced from Beyotime Institute of Biotechnology (Shanghai, China). DAT, VMAT2 and TH rabbit polyclonal antibodies were obtained from Santa Cruz Biotechnology, Inc (USA). MAO-B and β-actin rabbit antibodies for the standards were purchased from Abcam (England).

2.2. Treatment of animals

Sprague–Dawley (SD) male rats (6–7 weeks, 180–220 g) were obtained from Center of Laboratory Animals of Hubei Province, Wuhan, China. Individual weight was recorded and detailed physical examinations were performed during one week adaptation period to ensure animals met all the qualifications. All animals were single-caged, and given free access to commercial laboratory feed and tap water during the non-exposure period. In terms of results in pre-tests and previous reports which were carried out to assess neurotoxicity of ACR in rats, the dosage of 20, 30, 40 mg/kg/day were usually applied (Alturfan et al., 2012; Seale et al., 2012; Yu et al., 2013). They were randomly divided into four groups (10 rats per group): 0 (saline), 20, 30, 40 mg/kg/day. Animals were exposed to ACR through oral perfusion at the corresponding doses and the control group was given an equal volume of normal saline. Rats were continuously exposed to ACR for 20 days. Twenty four hours after the last ACR administration, all rats were sacrificed, and brain tissues were immediately removed, placed on ice and washed with pre-cooled PBS. Cerebellum, cerebral cortex and striatum were stripped immediately and stored at −80 °C for subsequent tests. The animal studies were performed in accordance with the guidelines for the care and use of laboratory animals, prepared by National Institute of Health, USA (Guide for the Care and Use of Laboratory Animals, 1996).

2.3. Behavior tests

2.3.1. Gait score

Gait score was determined by a previously described method (Zhu et al., 2008). The higher the score, the more difficultly the walk is. One point indicated that the rat was almost unaffected or normal; two points showed that the rat was slightly affected characterized by weakness, slight ataxia, liveliness, foot splay;

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