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Review

Inhalation of air polluted with gasoline vapours alters the levels of amino acid neurotransmitters in the cerebral cortex, hippocampus, and hypothalamus of the rat

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ABSTRACT

Background: This study was designed to investigate the impact of exposure to the vapours of two kinds of gasoline, a widely used fuel for the internal combustion engines on the levels of the amino acid neurotransmitters of the rat brain. Recent studies provide strong evidence for a causative role for traffic-related air pollution on morbidity outcomes as well as premature death (Health Effects Institute, 2009; Levy et al., 2010; von Stackelberg et al., 2013). Exposure to the vapours of gasoline or its constituents may be accidental, occupational by workers at fuel stations and factories, or through abuse as a mean of mood alteration (Fortenberry, 1985; Mc Garvey et al., 1999).

Two kinds of gasoline that are common in Egypt have been used in this study. The first contains octane enhancers in the form of lead derivatives (leaded gasoline; G1) and the other contains methyl-tertiary butyl ether (MTBE) as the octane enhancer (unleaded gasoline; G2). The levels of the major excitatory (aspartic acid and glutamic acid) and the inhibitory (GABA and glycine) amino acid neurotransmitters were determined in the cerebral cortex, hippocampus, and hypothalamus.

Results: The current study revealed that the acute inhalation of air polluted with the two types of gasoline vapours (1/2 LC_{50} for 30 min) induced elevation in the levels of aspartic and glutamic acids along with a decrease in glycine and GABA in most studied brain areas. Chronic inhalation of both types of gasoline (a single daily 30-min session of 1/5 LC_{50} for 60 days) caused a significant increase in the aspartic and glutamic acid concentrations of the hippocampus without affecting the levels of GABA or glycine.

Conclusion: Acute and chronic inhalation of either one of G1 and G2 vapours induced a disturbance and fluctuation in the levels of the free amino acids that act as excitatory and inhibitory neurotransmitters in the brain areas under investigation. These neurotransmitters are fundamental for the communicative functioning of the neurons and such effects may have a profound impact on the cognitive and sensorimotor functions of the brain resulting in serious psychological and physiological disorders..

Contents

Abbreviations: GABA, γ-aminobutyric acid; MTBE, methyl tertiary butyl ether; ETBE, ethyl tertiary butyl ether; TBA, tertiary butyl alcohol; TAME, tertiary amyl methyl ether; NMDA, N-methyl-D-aspartate.

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1. Introduction

Gasoline is a petroleum-derived liquid mixture consisting primarily of hydrocarbons and enhanced with additives to increase octane ratings, and improve performance in internal combustion engines. A number of previous studies have reported transient or persisting neurotoxic effects as a result of exposure of humans or animals to hydrocarbon fuels, or to certain constituent chemicals of these fuels (Health Effects Institute, 2009; Levy et al., 2010; von Stackelberg et al., 2013). The exposure may occur to raw fuel, vapour, aerosol, or fuel combustion exhaust by dermal, respiratory inhalation, or oral ingestion routes, and commonly occur concurrently with exposure to other chemicals and stressors (Ritchie et al., 2001). In the past, organic lead compounds were usually used as anti-knock/isooctane booster agents in gasoline. Organic tetraethyl lead is considered one of the gasoline constitutes as an octane enhancers (Goldings and Stewart, 1982). Tetraethyl lead itself is not toxic, but is converted in the body into triethyl lead which is water soluble and becomes concentrated in the brain where it induces several neurological changes. Triethyl lead is degraded to inorganic lead which interferes with the nerve conduction and explains the slowed nerve conduction velocity in cases of lead intoxication (Robinson, 1978). During the last two decades, lead derivatives have been almost completely replaced by "short-chain, oxygen containing hydrocarbons" such as methyl-tertiary-butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), tertiary butyl alcohol (TBA), and tertiary amyl methyl ether (TAME) as anti-knock agents in the production of unleaded gasoline and as substitutes for high octane aromatics in fuel (Schuetzle et al., 1994). It was suggested that addition of MTBE and ETBE to fuels improves combustion and produces less toxicity than that produced by the lead derivatives. It was reported that brain biogenic amines which comprises amino acid and monoamine neurotransmitters were altered with exposure to gasoline or one of its aromatic volatile constituents such as ethanol, benzene, and xylene (Ritchie et al., 2001; European Environment Agency, 2010; Westphal et al., 2010). The amplitude of effect varied with respect to gender and brain region (Chu et al., 2005). Also, Kinawy (2009) concluded that chronic exposure to either the leaded or the unleaded gasoline vapours impaired the levels of monoamine neurotransmitters and other related biochemical parameters in different brain areas and modulated several behavioural aspects related to aggression in rats. Some animal studies showed that acute inhalation produced behavioural changes that are concentration-dependent, reversible, and occur at concentrations lower than those necessary to produce explicit and irreversible neurotoxicity (Evans and Balster, 1991; Bowen et al., 2006).

The amino acid neurotransmitters of primary interest fall into two categories: the inhibitory amino acids which mainly comprise glycine and GABA and the excitatory amino acids which mainly comprise glutamate and aspartate. All of these compounds are present in high concentrations in the CNS and are extremely potent modifiers of neuronal and behavioural functioning.

Relatively little is known about the impact of gasoline abuse on the brain's amino acid neurotransmission systems and the results of such studies are usually inconsistent (Fortenberry, 1985; Mc Garvey et al., 1999). Therefore this study was designed to investigate the impact of inhalation of air polluted with leaded or unleaded gasoline vapours on these neurotransmitters in selected brain areas after acute, and chronic intoxication.

2. Materials and methods

2.1. Animals

Male Wister rats weighing (120–140 g) obtained from the Institute of Ophthalmic Disease Research were used and housed two per cage. Animals were kept in a controlled environment of 12 h light–dark cycle and $22 \pm 2\,^{\circ}C$ room temperature. Food and water were provided *ad libitum*. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

2.2. Experimental design

Ninety Male Wister rats were allocated into two experimental groups, acute and chronic, each of 30 rats. Each group was further divided into 3 subgroups, the first was exposed to the leaded gasoline and referred to as G1, the second to the unleaded gasoline and designated as G2 whereas the third group served as control. The exposure to gasoline-polluted air was conducted in a special apparatus designed and described by Ezzat et al. (2001). In this apparatus, the desired amount of gasoline was evaporated inside a chamber in a dynamic air flow system to ensure a constant concentration of the volatile pollutant during a fixed period of time. The control group was exposed to a gasoline-free air flow inside the exposure chamber under the same experimental conditions as the G1 and G2 groups. By using this apparatus, Ezzat et al. (2001) estimated the LC_{50} (30 min) to be 37,475 ppm for G1 and 39,928 for G2.

In the acute experiment, the G1 and G2 rats were exposed to a single session of the 1/2 LC_{50} of the leaded and unleaded gasoline respectively for 30 min.

In the chronic experiment, the G1 and G2 rats were exposed to the 1/5 LC_{50} dose for 30 min daily for the duration of 8 weeks.

At the end of the designated time for each experiment, the rats were sacrificed; the brain was excised and separated into the following areas: the cerebral cortex, hippocampus and hypothalamus. The brain tissue was stored frozen at $-20\,^{\circ}C$ pending analysis for the amino acid neurotransmitters by the HPLC.

2.2.1. HPLC determination of the free amino acid content in the brain tissue:

Brain free amino acids were determined by high performance liquid chromatography (HPLC) using the precolumn PTC derivatization technique according to the method of Heinrikson and Meredith (1984).

The levels of the tissue free glutamic acid, aspartic acid, GABA (γ-amino-butyric acid) and glycine were determined in the brain cortex, hippocampus and hypothalamus using a Perkin-Elmer HPLC system. This system comprises a quaternary pump; a column oven,