Environmental Toxicology and Pharmacology 30 (2010) 121–126

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

Metal deposition and functional neurotoxicity in rats after 3–6 weeks nasal exposure by two physicochemical forms of manganese

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ARTICLE INFO

Article history:
Received 14 December 2009
Accepted 27 April 2010
Available online 26 May 2010

Keywords:
Manganese
Nanoparticles
Manganese
Intranasal application
Neurotoxicity
Open field
Electrocorticogram
Evoked potentials
Rat

ABSTRACT

Airborne manganese represents a major risk of nervous system damage first of all in industrial settings. The resulting effects may depend on the dose and physicochemical form of Mn. To compare the effect of soluble and nanoparticulate Mn, adult male rats received daily instillation of $MnCl_2$ solution or MnO_2 nanoparticle suspension (dose: 2.53 mg Mn per rat) into the nasal cavity for 3 and 6 weeks. At the end of treatment, spontaneous open field motility was tested, electrophysiological recording was done in anesthesia, and brain tissue Mn level was determined. Metal level increase in the rats' brain, body weight gain reduction, and decrease of open field motility was significant in the $MnCl_2$, but not nano-Mn, treated rats. Most evoked cortical activity parameters were significantly altered in both groups, but spontaneous cortical activity spectrum only in the rats receiving $MnCl_2$. There was fair correlation between brain Mn levels and certain neuro-functional parameters, underlining the causal relationship. Electrophysiological tests might be more sensitive to the effects of Mn than general toxicological or neurobehavioral tests.

1. Introduction

Airborne manganese is, after sufficient long exposure or high dose, a well-known cause of health deterioration including damages to the nervous system. This is primarily an occupational hygienic problem in jobs like production and processing of Mn ores and the metal extracted, welding, dry cell battery manufacturing, etc. (ATSDR, 2000). The general public may be exposed due to the use of methylcyclopentadienyl manganese tricarbonyl as anti-knock petrol additive in certain countries (Lynam et al., 1999), organic Mn compounds applied as fungicides (Ferraz et al., 1988), and spent dry cells in incinerated solid household waste. Spray from drinking water may be the source of significant inhalational Mn exposure under certain circumstances (Elsner and Spangler, 2006).

Mn shows tropism to mitochondria-rich tissues including parts of the nervous system (Barceloux, 1999). Within the brain, Mn was detected after chronic exposure of humans in the basal ganglia, particularly in the globus pallidus, striatum and substantia nigra (Mergler, 1996; Lucchini et al., 1999). In rats, deposition in the hippocampus was reported (Takeda et al., 1998). Inhalation of Mn-containing airborne particles is especially hazardous because inhaled Mn can easily reach its target sites before biliary excretion (Elder et al., 2006; Fechter et al., 2002). Internal Mn doses much over the background were in fact observed in jobs involving Mn dust/fume exposure (Roels et al., 1997). Manganism, seen after lengthy (typically occupational) Mn exposure in humans, is a Parkinson-like syndrome with emotional, psychomotor, memory, etc. dysfunctions (Barceloux, 1999). Electrophysiological alterations in the victims also have been published (Sinczuk-Walczak et al., 2001; Sjögren et al., 1996).

To what extent inhaled particles and/or their Mn content can reach the CNS depends on the particles' characteristics and the physiological functions involved. Nanoparticles (NPs), in the submicron range, are a major part of metal fumes, and their effects can be distinct from those of larger (microscopic) particles. Due to their small size, high number concentration, and large specific surface area, NPs have greater biological activity per given mass than larger particles (Oberdörster et al., 2000, 2005), including oxidative stress induction, adsorption to organic molecules, and crossing tissue boundaries (Li et al., 2003; Stone et al., 2007).

Many of the NPs in the inhaled air are trapped in the nasopharynx (ICRP, 1994). The olfactory epithelium covers a considerable

Abbreviations: AUD, auditory; CAP, compound action potential; ECoG, electrocorticogram; EP, evoked potential; NP, nanoparticle; SS, somatosensory; VIS, visual.

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doi:10.1016/j.etap.2010.04.006

area within the nasal cavity, and the primary olfactory neurons provide a direct pathway by which foreign material may gain access to the central nervous system (Calderon-Garciduenas et al., 2002; Tjälve et al., 1996).

The consequences of chronic Mn exposure have been successfully modeled in animals (motor behavior: Normandin et al., 2004; Tapin et al., 2006; general evaluation: Gwiazda et al., 2007). In earlier works of our department, electrophysiological (spontaneous and evoked cortical activity) and behavioral (open field, acoustic startle response, etc.) effects were investigated by Vezér et al. (2005) with the water-soluble $MnCl_2$, administered orally to rats for several weeks. With respect to the emerging problem of environmental NPs and health, the scope of research was extended, so in the present work a water-soluble and an -insoluble (NP) form of Mn was given to rats, by instillation in the nasal cavity. The endpoints observed were tissue Mn levels, body and organ weights, open field behavior, and cortical and peripheral nervous electrical activity.

2. Materials and methods

2.1. Animals and treatment

Male Wistar rats were used, obtained from the University's breeding centre (age: ca. 6 weeks, body weight, ca. 140 g at start, see Fig. 2). The rats were housed in an air conditioned room maintained at 22 °C, with 12-h light/dark cycle (light on at 06:00) and free access to tap water and standard rodent chow. There were three groups of rats (*Con*, *Nano*, *Solute*, see below) with 16 animals each at start. The rats were treated every workday (i.e. five times a week) for 3 and 6 weeks (after 3 weeks, 8 of the 16 rats per group were killed and the remaining 8 were treated further on).

The animals received intranasal instillation of Mn in two physicochemically different forms: dissolved $MnCl_2$ (*Solute* group) and MnO_2 NPs (*Nano* group). Controls (*Con*) were vehicle treated. The vehicle used for dissolving $MnCl_2$ and suspending the NPs was a viscous "mucoadhesive" medium (1% Na hyaluronate, 10% polyethoxylated 40 hydrogenated castor oil, and 89% distilled water). $MnCl_2$ was of >99% purity, available commercially. The MnO_2 nanoparticles of ca. 23 nm mean diameter (Fig. 1) were synthesized at the Department of Applied Chemistry, University of Szeged Faculty of Science and Informatics, by a technique combining ultrasonic and hydrothermal treatment (for details, see Sárközi et al., 2009). The suspension was sonicated before, and repeatedly during, administration to counteract aggregation and sedimentation.

To perform intranasal instillation, the rats were briefly anesthetized with diethyl ether in a glass jar with air-tight lid. When the anesthesia was complete, the rat was laid on its back, and the material was administered into the left nasal cavity by means of a pipette tip, pulled out to ca. 0.2 mm tip diameter over a flame, and attached to a 100 μl Hamilton syringe. The dose was based, initially, on literature data (Henriksson et al., 1999) showing that ca. 1000 μg of Mn, given intranasally, can cause histological alterations. The technical limits of available concentration of the nanosuspension and of the volume that could be instilled in the nasal cavity (40 μl) determined finally the dose of 2.53 mg Mn per rat, both from the NPs and from dissolved $MnCl_2$. The rats were weighed before each Mn administration and were regularly checked for general toxic symptoms. All other investigations were performed at the end of the 3 or 6 weeks treatment period.

2.2. Behavioral investigation

On the day following the last administration, spontaneous motor activity was tested using an open field (OF) box of 48 cm × 48 cm × 40 cm size. The animals were placed individually into the center of the box, and the instrument was recording their horizontal and vertical motor activity in 10 min sessions, by means of grids of infrared gates. A more than 40 mm shift in the location of the interrupted infrared beams during the time resolution unit of 1 s was interpreted as ambulatory movement (running); less shift, as local activity (grooming); and no shift, as immobility. Rearing was detected by another grid at 12 cm above the floor. From these data, counts, time and run length of the activity forms were calculated. OF tests were done always between 10:00 and 14:00.

2.3. Electrophysiological investigation

The next day, the rats were anesthetized with urethane (1000 mg/kg ip.; Mook, 2006). Their head was fixed in a stereotaxic frame, the skull was opened and the left hemisphere was exposed. The wounds were sprayed with 10% lidocaine, and the exposed dura was protected by a thin layer of petroleum jelly. After 30 min recovery, silver electrodes were placed on the primary somatosensory (SS), visual (VIS) and auditory (AUD) areas. Electrocorticogram (ECoG) was recorded from these areas for 6 min. Then, the same electrodes were used to record sensory evoked potentials

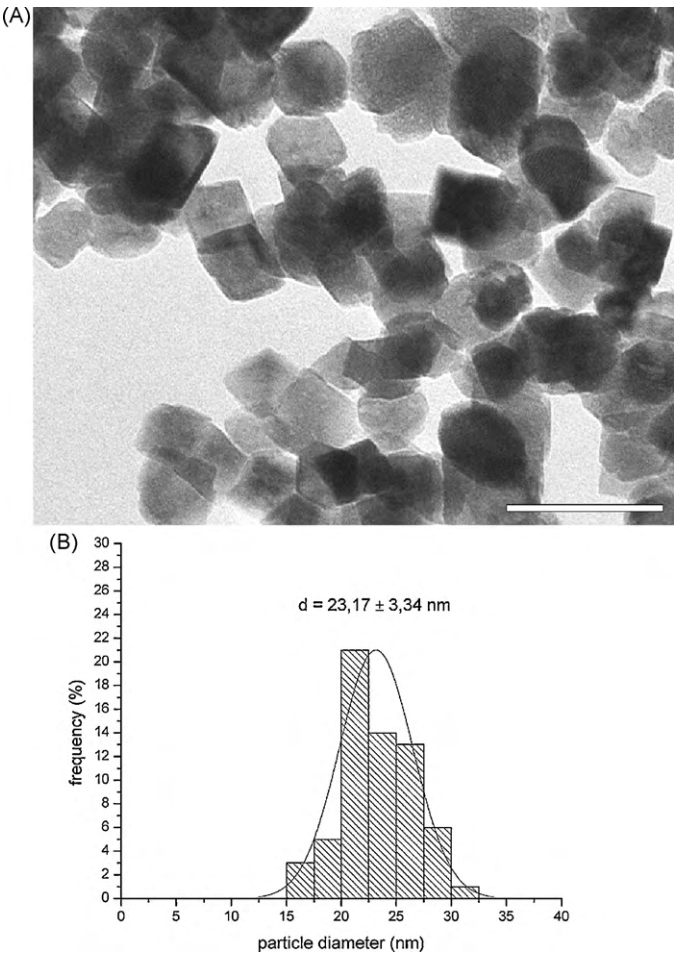

Fig. 1. Transmission electron micrograph of the MnO_2 nanoparticles (A, scalebar: 50 nm); size distribution of the particles (B, determined by X-ray diffraction).

(EPs). For somatosensory stimulation, two needles were inserted into the contralateral whiskery skin to deliver square electric pulses (3–4 V, 0.05 ms, 1, 2 and 10 Hz). Visual stimulation was produced by a high-luminance white LED aimed directly at the rat's right eye, driven by 0.2 ms pulses at 1 Hz. The acoustic stimuli were clicks (1 Hz, 40 dB) guided from a miniature earphone into the animal's right ear via the hollow ear bar. One train of fifty stimuli of each modality per rat were applied.

Finally, compound action potential (CAP) form the rat's tail nerve was recorded. Two stimulating needles (delivering 4–5 V, 0.05 ms pulses at 1, 20 and 50 Hz) were inserted into the tail base; and another two, for recording, 50 mm distally.

From the ECoG records, the relative spectral power by frequency bands (delta, theta, alpha, beta1, beta2, gamma; standard human EEG bands as described in Kandel and Schwartz, 1985) was determined. The EPs and tail nerve CAPs were averaged, and their latency and duration was measured manually. The change of latency of the somatosensory EP, and change of latency and amplitude of the CAP, with increasing stimulation frequency was also investigated as a possible indicator of the action of the treatment on the state of the cortex (Papp et al., 2004). With double-pulse tail stimulation, the length of the refractory period of the tail nerve was determined. From the CAP latency data, conduction velocity of the nerve was calculated, and from that, refractory period (absolute refractory period was defined as the time span after a nerve discharge within which no second stimulation is possible, while within the subsequent relative refractory period the stimulus sensitivity of the nerve is partially restored). All electrophysiological recording and analysis was done by means of the Neurosys 1.11 software (Experimetria Ltd., Budapest, Hungary).

2.4. General toxicology and Mn level determination

The rats were at the end killed by an overdose of urethane, dissected, and the organ weight of the brain, liver, lungs, heart, kidneys, spleen, thymus and adrenals was measured. From these data, relative weights were calculated by relating organ weights to brain weight. Brain weight was used as reference (Schärer, 1977) because (in contrast to body weight) it was minimally affected by the treatment. Brain samples were taken, and Mn level was determined by acidic digestion and GC–MS.