

NEUROBEHAVIORAL FUNCTIONS, SERUM PROLACTIN AND PLASMA RENIN ACTIVITY OF MANGANESE-EXPOSED WORKERS

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Objective of this study was to assess effects of manganese (Mn) exposure on 56 workers employed in a Mn welding workshop of a machine building factory in Taiyuan (Shanxi Province, P.R. China) for a mean period of 16.1 years. The mean air Mn level in the workshop was 138.4 $\mu\text{g}/\text{m}^3$. Neurobehavioral Core Test Battery (NCTB), including the Profile of Mood States (POMS), was performed. Blood pressure (BP) increase following immediate stand-up (BP-IS), serum prolactin (PRL) and plasma renin activity (PRA) in supine position were also determined. Most of the NCTB scores of the Mn-exposed workers were lower than those of the controls, while the POMS scores were higher, indicating a Mn-induced impairment of neurophysiological functions and a deflection of mood towards negative emotion states. PRL values of the Mn-exposed workers were higher than those of the controls. BP-IS of Mn-exposed workers was significantly lower than that of the controls. PRA of the same workers was augmented more than 200 %. In the Mn-exposed workers, the higher PRL values are possibly due to a reduced inhibitory effect on pituitary lactotrope cells by the tubero-infundibular dopamine system; the decreased BP-IS was referred to imbalance between the sympathetic and parasympathetic activities, whereas the higher basal PRA was thought to depend on neuroendocrine changes (including increased central sympathetic tone) and/or on a direct effect of Mn on renal juxta-glomerular cells. On the whole, this study demonstrates that occupational Mn exposure is responsible for neurobehavioral changes coexisting with alterations of neuroendocrine and humoral systems.

Manganese (Mn) is a ubiquitous constituent of the environment comprising about 0.1 % of the earth crust. Today, occupational exposure to Mn occurs during several activities including arc welding. High levels of Mn in air, water and soil, which are able to affect human health, may depend on pollution produced by working activities and/or derive from geological sources (1). Moreover, methylcyclopentadienyl Mn was used in Canada since 1976 as an additive anti-knock agent in unleaded gasoline (2).

Mn is considered an essential element required for the human diet (3). It also acts as a component of several metallo-enzymes including superoxide dismutase, a scavenger of reactive oxygen species (ROS) (4). Mn is known to catalyze autoxidation of dopamine (DA), in the presence of L-cysteine, in buffered solution (5). Within its selective neurotoxicity for DA neurons of the central nervous system (6), Mn has been implicated in degeneration of the nigrostriatal dopaminergic neurons in idiopathic and chemically-induced Parkinson's

Key words: manganese, neurobehavioral function, prolactin, plasma renin activity, sympathetic system

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disease. Such selectivity was found to depend upon interactions of Mn^{++} with the DA transport system in these neurons (7). Mn (along with iron) acts as a pro-oxidative agent at synaptic level. Mn^{++} also appears to influence Ca^{++} transport kinetics in the mitochondria, thus leading to defective mitochondrial function, decreased oxidative phosphorylation and ATP production, and accumulation of ROS (8). Moreover, intrathecal administration of $MnCl_2$ to young male rats caused DA depletion in the caudate-putamen correlating with a decrease in spontaneous motor activity (9). In aged rats, Mn exposure was able to increase formation of ROS more than in young rats and an age-related impairment of the neuronal antioxidant systems was suggested to play an enabling role in Mn neurotoxicity (10).

Manganism is a central nervous system disease first described in the 1800s following exposure to high concentrations of Mn oxides (11). On the other hand, Mn exposure can cause a Parkinsonian syndrome within one or two years. This syndrome includes gait disorders and postural instability, psychiatric disorders with compulsive behavior, emotional lability and autonomic dysfunction (12-14). In this regard, Mn-exposed workers may present not only reduction of motor functions but also increased olfactory perceptions (15). Since increased serum prolactin (PRL) is a common finding among Mn-exposed subjects, the assay of PRL has been proposed, as a neuroendocrine biomarker, to assess the effect of this metal on the tubero-infundibular DA system, which exerts a tonic inhibitory effect on the pituitary lactotrope cells (16,17).

Although Mn was found *in vitro* to play a protective role against free radicals in ventricular myocytes (18), other *in vitro* and *ex vivo* assays as well as *in vivo* acute and subacute toxicity tests evidenced cardiotoxic effects of the metal (19). In this respect, higher incidence of electrocardiographic abnormalities in Mn-exposed workers was reported (20).

There are only few and controversial studies in humans on neurogenic, neurohumoral and autacoidal mechanisms regulating cardiovascular homeostasis and BP levels. This study, therefore, aimed to investigate the relationship between the effects of Mn exposure on neurobehavioral parameters and those on neuroendocrine and humoral (autacoidal) pathways.

MATERIALS AND METHODS

A Mn welding workshop of a machine building factory was investigated. The ventilation of the premises was efficient. Air Mn samples were collected by air sampler in the welding operation sites at the highness of workers' breathing zone (about 160 cm) with flow speed 15 L/min for 10 minutes. 20 samples were collected. The air Mn, Cd, Ni and Fe concentration were measured by graphite furnace atomic absorption spectrophotometry according to Wang (21). 56 welders (Mn-exposed) men and 34 control men were recruited for the study. Welding workers had been exposed to Mn for (mean \pm SEM) 16.17 ± 0.73 years (range 3 -32 years) working for 4 hours per day and 5 days per week. Subjects of the two groups had similar age, family income, smoking habit (22 and 24 %, respectively) and education degree. Subjects suffering from diseases, in treatment with drugs, taking a large amount of alcohol (>500 mL ethanol/week) and smoking more than 40 cigarettes/day were excluded from the study. The age of the Mn-exposed subjects was (mean \pm SEM) 33.7 ± 0.6 years and that of controls 33.2 ± 0.9 years. All the examined subjects were living in the same area of the South District of Taiyuan City (Shanxi Province, P.R. China) with similar exposure to urban environment [recently, we found mean blood lead (Pb) levels of 12 mg/dL in the women resident in this area (22)].

The Mn-exposed workers and the control subjects attended the hospital in the early morning, in fasting condition, after a sleeping night. The neurobehavioral tests were performed in a clinic room with the same examiner, from 8.30 to 10.30 a.m., lasting about 30 minutes for each subject. The clinic room was quiet, illuminated with a daylight lamp, and the temperature in the room was 20-22° C.

The WHO recommended Neurobehavioral Core Test Battery (NCTB) was used for interviewing all the examined subjects (23). NCTB is composed of two parts. One part is the inventory of Profile of Mood States (POMS) questionnaire including Anger-Hostility (POMSA), Confusion-Bewilderment (POMSC), Depression-Dejection (POMSD), Fatigue-Inertia (POMSF), Tension-Anxiety (POMST), and Vigor-Activity (POMSV), which reflect neuropsychological variations caused by neurotoxicants; the other part is a test battery including six tests: Simple Reaction Time (SRT), which reflects response speed and attention; Digit Span (DSP), which reflects attention and auditory memory; Santa Ana Dexterity (SAN), which presents

manual coordination and speed; Digit Symbol (DSY), which shows cognition and motor speed; Benton Visual Retention (BVR), which reflects visual cognition and memory, coordination and speed; and Pursuit Aiming II (PA), which presents manual coordination and speed. SRT is divided into SRTF (fastest simple reaction time) and SRTS (slowest simple reaction time); DSP is divided into DSPF (forward) and DSPB (backward); SAN includes SANP (preferred hand) and SANN (non-preferred hand); PA includes PAC (correct) and PAE (erroneous).

Serum PRL and plasma renin activity (PRA) and BP increase following immediate standing-up (BP-IS) were determined in 21 Mn exposed workers and 16 control men. These 21 Mn-exposed workers and 16 controls were included in the previous groups of 56 Mn-exposed workers and 34 controls. The age and the smoking habit of 21 Mn-workers and 16 controls were similar to those of the groups composed of 56 workers and 34 controls. After a sleeping night, in fasting condition, the recruited subjects were implanted with a needle (connected to a heparinized cannula), which was inserted into a forearm vein. Afterwards, blood samples were collected after 30 minutes of standing in supine position. Serum prolactin (PRL) and plasma renin activity (PRA) were determined by radioimmunoassay according to Garden and Weintraub (24).

BP-IS was determined from 8.30 to 9.30 a.m.; the recruited men remained in supine position for 5 minutes

before testing BP and then stood up quickly. BP-IS was the difference in systolic BP during lying down and after standing up; the value of BP-IS (mmHg) was the mean of 3 determinations with 5 minutes of interval; general procedures were in accordance with Ewing (25).

Kolmogorov-Sminorv tests showed that the groups of 56 workers and 34 controls conformed more the normal distribution than the smaller groups of 21 workers and 16 controls. Therefore, parametric test were utilized in the larger groups and non-parametric (Mann-Whitney U-test) in the smaller ones.

Data were compared by analysis of covariance in order to exclude the influences of age, family income, smoking habit and education. All sets of data were expressed as means \pm SEM, P values less than 0.05 were considered as significant.

RESULTS

Air Mn level in the premises of the factory was (mean \pm SEM) $138.4 \pm 11.6 \mu\text{g}/\text{m}^3$ (range 38-198; determinations n 20); air Cd was $12.6 \pm 2.8 \text{ ng}/\text{m}^3$ (range 3.0-48.3); air Fe was $581 \pm 45 \mu\text{g}/\text{m}^3$ (range 354-950); air Ni was $3.8 \pm 0.53 \mu\text{g}/\text{m}^3$ (range 1.2-8.7).

The accumulative exposure of Mn, Cd, Fe and Ni were $2.23 \pm 0.19 \mu\text{g}/\text{m}^3 \cdot \text{year}$, $0.204 \pm 0.046 \mu\text{g}/\text{m}^3 \cdot \text{year}$, $9.40 \pm 0.73 \text{ mg}/\text{m}^3 \cdot \text{year}$ and $0.062 \pm 0.08 \text{ mg}/\text{m}^3 \cdot \text{year}$, respectively.

Items	Mn-exposed (n = 56)	Controls (n = 34)	F	p
SRT	242.58 \pm 6.21	325.85 \pm 26.48	3.578	0.001**
SRTF	172.57 \pm 7.78	227.79 \pm 21.89	2.054	0.050*
SRTS	466.16 \pm 30.69	563.50 \pm 56.98	2.205	0.054
DSP	14.55 \pm 0.39	18.50 \pm 0.68	2.758	0.010*
DSPF	9.64 \pm 0.26	12.67 \pm 0.44	2.559	0.015*
DSPB	4.91 \pm 0.21	5.82 \pm 0.37	2.038	0.052
SANP	42.92 \pm 0.85	41.79 \pm 1.13	0.051	0.846
SANN	39.92 \pm 0.67	40.00 \pm 1.46	0.555	0.811
DSY	41.55 \pm 1.66	50.38 \pm 2.03	4.172	0.001**
BVR	7.12 \pm 0.16	6.85 \pm 0.41	0.426	0.902
PA	203.88 \pm 4.68	220.08 \pm 5.20	3.882	0.001**
PAC	203.46 \pm 4.22	226.23 \pm 5.85	3.798	0.001**
PAE	4.33 \pm 0.94	3.97 \pm 1.33	1.278	0.267

Tab. I. Neurobehavioral tests of Mn-exposed workers and controls

Values (mean \pm SEM) represent the scores obtained for each item of the Neurobehavioral Core Test Battery (NCTB). Scores were processed by covariance analysis in order to exclude the influence of age, family income, smoking habit and education degree. ns: not significant. Difference statistically significant: * $p < 0.05$, ** $p < 0.01$. SRT: Simple Reaction Time; SRTF: Fastest SRT; SRTS: Slowest SRT; DSP: Digit Span; DSPF: DSP (forward); DSPB: DSP (backward); SANP: Santa Ana Dexterity (preferred hand); SANN: Santa Ana Dexterity (non-preferred hand); DSY: Digit Symbol; BVR: Benton Visual Retention; PA: Pursuit Aiming II; PAC: PA (correct); PAE: PA (erroneous)

Tab. II. Profile of mood states (POMS) of Mn-exposed workers and controls

Items	Mn-exposed (n=56)	Controls (n=34)	F	p	
POMSA	13.75 ± 1.10	12.50 ± 1.40	1.258	0.277	<i>Values (mean ± SEM) represent the scores obtained for each item of the Profile of mood states (POMS). Scores were processed by covariance analysis in order to exclude the influence of age, family income, smoking habit and education degree. ns: not significant. Difference statistically significant: *p<0.05, **p<0.01</i> POMSA: Profile of Mood State (POMS)/Anger-Hostility; POMSC: POMS/Confusion-Bewilderment; POMSD: POMS/Depression-Dejection; POMSF: POMS/Fatigue-Inertia, POMST: POMS/Tension-Anxiety; POMSV: POMS/Vigor-Activity.
POMSC	9.48 ± 0.48	6.90 ± 1.16	2.484	0.018*	
POMSD	16.50 ± 1.04	12.82 ± 1.12	2.634	0.013*	
POMSF	8.70 ± 0.65	6.18 ± 0.46	2.976	0.006**	
POMST	12.84 ± 0.82	9.21 ± 0.91	4.075	0.001**	
POMSV	16.32 ± 0.89	16.09 ± 1.98	1.380	0.218	

Tab. III. Serum prolactin (PRL) and plasma renin activity (PRA) of Mn-exposed workers and controls

	Mn-exposed (n=21)	Controls (n=16)	p	
PRL (mg/L)	21.75 ± 0.55	17.01 ± 0.47	<0.05	<i>Values are expressed as mean ± SEM. Data are processed by Mann-Whitney U test</i>
PRA (ng/mL/h)	3.17 ± 0.22	1.49 ± 0.19	<0.01	

Results of the neurobehavioral tests are listed in Tab. I. The scores of SRT, SRTF, DSP, DSPF, DSY, PA and PAC of the Mn-exposed workers were significantly lower than those of the control subjects. Moreover, in the Mn-exposed workers, also the scores of SRTS and DSPB were lower than those of the controls, but only with a borderline statistical significant difference, while SANP, SANN, BVR and PAE were similar to those of the controls.

The results of POMS are reported in Tab. II. The scores of POMSC, POMSD, and, mostly, POMSF, POMST, of the Mn-exposed workers were significantly higher than those of the controls. However, POMSA and POMSV of the Mn-exposed men did not show significant changes.

BP-IS of the Mn-exposed workers was 5.6 ± 0.5 mmHg, while that of the controls was 8.0 ± 0.9 mmHg ($p < 0.05$ processed by Mann-Whitney U test).

Serum PRL and PRA values of the Mn-exposed workers were significantly augmented in relation to those of the control men (Tab. III).

DISCUSSION

The welding workers had been exposed to Mn for long time (mean 16.1 years). When the workers were investigated, mean air Mn concentration in the factory was $138.4 \mu\text{g}/\text{m}^3$. However, a previous determination in some premises of the factory showed mean values exceeding $200 \mu\text{g}/\text{m}^3$. It may thus not be excluded that Mn exposure of the workers had been recently reduced by environmental improvements.

Welding workers were exposed to very low Cd levels (mean $12.6 \mu\text{g}/\text{m}^3$) able to affect neither renal mechanisms of cardiovascular regulation or nervous functions (26). Moreover, the levels of occupational Ni and Fe exposure were not harmful for human health. Therefore, only Mn exposure was able to affect the nervous and cardiovascular parameters of the investigated workers.

Neurobehavioral tests measure integrated activities of the nervous system. Such tests are considered very sensitive functional indicators of neurotoxicity, being the behavioral performances

the expression of sensory, motor, neuroendocrine, neurovegetative and integrative processes involving both central and peripheral nervous system (27,28). The sensitivity, reliability, validity and cross-cultural consistency of NCTB as an integrated battery have been confirmed, with some disparity of different tests dependent on cultural groups, and with some influence of age and education degree (Anger et al., 1997). In this regard, many reports on neurobehavioral assessment for neurotoxicity of chemicals have been published, mostly dealing with the exposure to Pb, Mn, aluminum, organic solvents and pesticides (21,23,29-31).

In this study, the majority of the scores of NCTB (neurobehavioral tests and POMS) was significantly altered by Mn exposure. On the basis of these results, Mn appears to represent, at levels of occupational exposure, a factor of neurobehavioral impairment. This impairment involves reduced performances of neurophysiological functions (including decreased attention and auditory memory, cognition, manual coordination and performance speed) and deflection of the mood state toward depressive, anxious and asthenic components [as indicated by the elevated scores of confusion-bewilderment (POMSC), depression-dejection (POMSD), fatigue-inertia (POMSF) and tension-anxiety (POMST) in the Mn-exposed men]. However, Mn did not affect active emotion/reaction scores [anger-hostility (POMSA), vigor-activity (POMSV)], thus appearing that Mn-exposure affects quite specifically the neuropsychological determinants of mood state. In this respect, animal experiments showed the emotionally negative states to be induced by hyperactivity of serotonergic pathways and/or hypoactivity of noradrenergic, dopaminergic and opioid ones, while the emotionally positive states result from opposite activities of these central neurogenic systems; moreover, the serotonergic, noradrenergic and dopaminergic systems play a central role in the processes of reinforcement, acquisition of the evaluative capacities and realization of all types of behaviour (32). On the other hand, PRL, an index of functionality of the hypothalamo-pituitary axis, is regarded as a modulator of immunity (33,34) and behavior showing anxiolytic and anti-stress effects (35). Therefore, a relationship between higher values of

serum PRL and impairment of neurophysiological function (with shift towards emotionally negative states) of the Mn-exposed workers appears to be suggestive. On the other hand, the observed higher levels of serum PRL are in agreement with the results of previous studies, in which increased serum PRL was a common finding among subjects exposed to Mn. The involved mechanism in the serum PRL increase was supposed to consist in a Mn inhibiting effect on the tubero-infundibular DA system, in turn inhibiting production and/or release of PRL from the pituitary lactotrope cells (16,17,36).

Autonomic nervous system function test battery recommended by Ewing (1993), including BP-IS, was used not only on patients (in clinical studies) but also on non-symptomatic workers exposed to neurotoxic agents (30,37). This study showed that BP-IS of the Mn-exposed workers was significantly reduced, thus evidencing that sympathetic and/or parasympathetic activities may be affected by Mn exposure. Our results confirm those of Barrington et al. (20) who found decreased chronotropic reactivity, depending on sympathetic-vagal imbalance, in eight Mn-exposed workers by using the 24 hr-electrocardiographic monitoring (Holter).

There are no data on the effects of Mn on the renal juxta-glomerular (JG) apparatus and renin-angiotensin-aldosterone (RAA) system for explaining the increased PRA of the Mn-exposed workers. This apparatus, with a rich sympathetic nerve supply, is composed of macula densa and modified granulated smooth muscle cells of the afferent arteriole of the glomerulus, with a minor contribution by those of the afferent arteriole and by interstitial cells (38). The JG apparatus has baroreceptors in the arterioles, responding to change of transmural pressure gradient, and chemoreceptors in the macula densa, detecting changes in the composition of fluids of the distal tubule. The secretion of renin is stimulated by changes in sympathetic activity, modification of body position, reduction of renal perfusion pressure, extracellular volume depletion, changes in plasma levels of electrolytes (such as sodium, potassium and calcium) and circulating hormones. In particular, the release of renin from JG cells is stimulated by substances (such as b_1 - and b_2 - adrenoreceptor agonists) leading to an increase of the intracellular concentration of cyclic adenosinemonophosphate

(cAMP). The levels of intracellular free calcium in the JG cells and the transmembrane sodium gradient seem to play a major role in the release of renin (39). Moreover, it is known that DA-1 and DA-2 receptor subtypes are localized in various regions within the kidney (lacking DA neurons), including vasculature (DA-1) and sympathetic nerve endings in blood vessels (DA-2). Activation of the DA-1 and DA-2 receptors (by circulating DA and by DA released from renal sympathetic endings) involves stimulation of adenylate cyclase activity and inhibition of the inositol 1,4,5-triphosphate-dependent Ca^{++} release, respectively, with inhibition of sodium reabsorption from tubules and consequent increased natriuresis (40,41). DA causes vasodilation in the renal circulation, suppresses aldosterone secretion, directly stimulates renal sodium excretion, suppresses noradrenaline release at the sympathetic nerve terminals by a pre-synaptic inhibitory mechanism, and influences the secretion of renin. Therefore, considering the selectivity of Mn in affecting catecholamine (DA) neurogenic systems and metabolic pathways (42), the marked PRA increase of the Mn-exposed workers is likely to depend on Mn-induced alterations of catecholamine (DA) metabolism and/or adrenergic neurotransmission in the kidney. However, effects of Mn on other (renal) systems and pathways, involved in renin production and release (e.g., the kallikrein-kinin and nitric oxide ones) and interacting with the catecholamine ones, cannot be excluded (41,43).

Neuroendocrine, immune, humoral and renal mechanisms (responsible for Pb-induced arterial hypertension) have been described in previous studies on Pb-exposed workers (21,44). In this respect, workers with a short period of Pb exposure showed high increase of plasma renin activity (PRA) in response to the change of position, while there was a low PRA response and high PRA values in supine position in workers suffering from blood hypertension and/or nephrosclerosis with prolonged Pb exposure (45,46). The results of these studies on Pb-exposed workers suggest that the reduced BP increment with the change of position, along with the increased PRA in supine position of the Mn-exposed workers, may in part be explained by an increased central sympathetic tone and/or by an increased response following activation of the JG adrenergic receptors.

It is known that Mn environmental exposure is a better marker of exposure to the metal than blood or urinary Mn (14). The mean air concentration of Mn in the premises of the factory of the exposed workers was recently $138.4 \mu\text{g}/\text{m}^3$. However, workers may have been previously exposed to higher Mn levels. This study implies that workers exposed to these levels of air Mn showed adverse health effect. The adequate health-based evidence that became available in the 1990s induced the ACGIH to adopt the current TLV-TWA of $200 \mu\text{g}/\text{m}^3$ (as Mn in total dust) in 1995 (47). However, there are still higher standards for occupational Mn exposure among several jurisdictions including those of US-OSHA, Germany, Sweden and Australia.

In conclusion, this study demonstrates that Mn exposure induces neuroendocrine effects including modifications of behavioral tests and increased PRL. Moreover, Mn exposure appears to change the sympathetic-vagal balance and affect renal mechanisms involved in BP regulation. In particular, the Mn-induced PRA increase may be referred to neuroendocrine changes and/or to direct renal effects of the metal on the JG apparatus and tubular cells.

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