NeuroToxicology 54 (2016) 170–177

Contents lists available at ScienceDirect

NeuroToxicology

Full length article

Lead toxicity promotes autonomic dysfunction with increased chemoreceptor sensitivity

Vera Geraldes[a,b], Mafalda Carvalho[b], Nataniel Goncalves-Rosa[a,b], Cristiano Tavares[a,b], Sérgio Laranjo[b], Isabel Rocha[a,b,*]

[a] Institute of Physiology, Faculty of Medicine of the University of Lisbon, Av. Prof Egas Moniz, 1649-028 Lisbon, Portugal
[b] Cardiovascular Centre of the University of Lisbon, Av. Prof Egas Moniz, 1649-028 Lisbon, Portugal

ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form 11 April 2016
Accepted 26 April 2016
Available online 28 April 2016

Keywords:
Lead toxicity
Sympathoexcitation
Hypertension
Chemoreceptor reflex
Baroreceptor reflex
A-779
PVN-NTS axis

ABSTRACT

Mortality and morbidity by toxic metals is an important issue of occupational health. Lead is an ubiquitous heavy metal in our environment despite having no physiological role in biological systems. Being an homeostatic controller is expected that the autonomic nervous system would show a degree of impairment in lead toxicity. In fact, sympathoexcitation associated to high blood pressure and tachypnea has been described together with baroreflex dysfunction. However, the mechanisms underlying the autonomic dysfunction and the interplay between baro- and chemoreflex are not yet fully clarified. The angiotensinogenic PVN-NTS axis (paraventricular nucleus of the hypothalamus – nucleus tractus solitarius axis) is a particularly important neuronal pathway that could be responsible for the autonomic dysfunction and the cardiorespiratory impairment in lead toxicity. Within the current work, we addressed in vivo, baro- and chemoreceptor reflex behaviour, before and after central angiotensin inhibition, in order to better understand the cardiorespiratory autonomic mechanisms underlying the toxic effects of long-term lead exposure. For that, arterial pressure, heart rate, respiratory rate, sympathetic and parasympathetic activity and baro- and chemoreceptor reflex profiles of anaesthetized young adult rats exposed to lead, from foetal period to adulthood, were evaluated. Results showed increased chemosensitivity together with baroreceptor reflex impairment, sympathetic over-excitation, hypertension and tachypnea. Chemosensitivity and sympathetic overexcitation were reversed towards normality values by NTS treatment with A-779, an angiotensin (1–7) antagonist. No parasympathetic changes were observed before and after A-799 treatment. In conclusion, angiotensin (1–7) at NTS level is involved in the autonomic dysfunction observed in lead toxicity. The increased sensitivity of chemoreceptor reflex expresses the clear impairment of autonomic outflow to the cardiovascular and respiratory systems induced by putative persistent, long duration, alert reaction evoked by the long term exposure to lead toxic effects. The present study brings new insights on the central mechanisms implicated in the autonomic dysfunction induced by lead exposure which are relevant for the development of additional therapeutic options to tackle lead toxicity symptoms.

1. Introduction

Lead (Pb) is a toxic heavy metal used in many industrial activities and a common environmental contaminant widely distributed around the world. It is well documented that lead can cause several adverse health effects. The cardiotoxic manifestations include cardiac electrical disturbances and signs of vascular degeneration, abnormal vascular smooth muscle function and altered vessel compliance (Navas-Acien et al., 2007; Vaziri, 2008; Gump et al., 2011). In rats chronically exposed to lead, high blood pressure values were detected induced by positive inotropism and increases in total peripheral resistance, without chronotropic changes (Boscolo and Carmignani, 1988; Gump et al., 2011). More studies have found correlations among occupational lead exposure hypertension and renal function impairment, as kidney is not only a primary organ for lead toxicity but indirect cardiovascular effects could occur secondarily to renal injury (Schwartz et al., 1988; Weaver et al., 2005, 2008; Poręba et al., 2011). Also, neurotoxicity and respiratory disease were described in humans, particularly in lead miners occupationally exposed to lead (Masjedi

* Corresponding author at: Instituto de Fisiologia, Faculdade de Medicina de Lisboa, Av. Professor Egas Moniz, Ed. Egas Moniz, Piso 1, 1649-028 Lisbon, Portugal.
E-mail address: isabelrocha0@gmail.com (I. Rocha).

http://dx.doi.org/10.1016/j.neuro.2016.04.016
0161-813X/

et al., 1989; Abadin et al., 2007). Animal and human studies reported impairment of cognitive capabilities associated to a disruption of the cholinergic hippocampal system (Bourjeily and Suszkiw, 1997; Gump et al., 2011).

Although the autonomic nervous system is not typically viewed as a primary target of lead toxicity, impairment of cardiovascular and respiratory regulation has already been described despite some suggestions that none of the autonomic indicators, like electrocardiographic R–R interval variations, were correlated with lead exposure (Gennart et al., 1992). In fact, it has been reported autonomic dysfunction caused by lead, affecting predominantly the parasympathetic component of the autonomic nervous system (Murata and Araki, 1991; Teruya et al., 1991; Artamonova et al., 1998; Madan et al., 2007). Moreover, in a paediatric study was shown that lead-blood levels were associated with increasing co-inhibition of sympathetic and parasympathetic activation during acute psychological stress (Gump et al., 2011). Lead has, also, the ability to reduce baroreflex sensitivity, vagal parasympathetic tone and to increase sympathetic activity by central mechanisms, which are not yet clarified but could include impairment of cholinergic and dopaminergic transmission and lead induced oxidative stress (Bielarczyk et al., 1996; Bourjeily and Suszkiw, 1997; Brockel and Cory-Slechta, 1999). This triad of effects – baroreflex hyposensitivity, decreased parasympathetic tone and sympathetic over-excitation – has been observed in several pathologies as essential hypertension, heart failure or acute cardiac ischemia. In these conditions was hypothesized that an initial putative noxious stimulus triggers an alert reaction which is initially protective but, with time, evolves for a persistent and deleterious sympathoexcitation. In particular for hypertension, several studies have pointed out that the persistent increase in sympathetic tone is a major contributor to, both, its initiation and maintenance (Yamada et al., 1988; Rocha et al., 2003a,b; Grassi, 2004; Smith et al., 2004; Guyenet and Guyenet, 2006; Fisher and Paton, 2012; Geraldes et al., 2014a,b). Likewise, in acute heart ischemia, an enhancement of the reflex cardiovascular responses to chemoreceptor reflex and a depression of baroreceptor reflexes can also account for the characteristic autonomic imbalance of this condition (Rocha et al., 2003a,b).

The mechanisms underlying the autonomic dysfunction and the interplay between baro- and chemoreflex are not yet fully clarified but they could involve specific central pathways namely those that link the paraventricular nucleus of the hypothalamus (PVN), where the alert reaction initiates, with lower brainstem regions where primary reflex information from peripheral sensitive receptors is integrated, eg. the nucleus of solitary tract (NTS) (Silva-Carvalho et al., 1995; Rocha et al., 2003a,b; Geraldes et al., 2014b). In particular, an angiotensinogenic pathway seems to be involved in the modulation of these physiological responses (Diz et al., 2008a,b; Paton et al., 2008; Rosário et al., 2003). Thus, the current work has sought to address, in an animal model of chronic lead exposure, baro- and chemoreceptor reflex behaviour, before and after central angiotensin inhibition, in order to better understand the cardiorespiratory autonomic mechanisms underlying the toxic effects of long-term lead exposure.

2. Methods

2.1. Development of the animal model of chronic lead exposure

Seven day pregnant *Wistar* rats (n = 6) were divided into lead treatment and control groups. In the lead treatment group, the tap drinking water was replaced with 0.2% (p/v) solution of lead acetate. After been weaned at 21 days, rat pups continued divided into two groups in accordance to their mothers previous type of drinking: lead-exposed pups (Pb-rats) and tap water for control pups (Ctl-rats) (n = 14/group). The lead exposure regimen was chosen based on previous studies and took into account that ingestion is one of the three main intake routes for lead absorption by the body (Lefauconnier et al., 1983; Bielarczyk et al., 1996).

After being absorbed, lead is distributed along the body being bounded not only to red blood cells but also to bone and soft tissues. To confirm the effectiveness of the chosen exposure route, lead presence in the bone was evaluated, as stated elsewhere (Guimarães et al., 2012). In brief, 11 weeks animals' (n = 3/group) were sacrificed with an overdose of sodium pentobarbitone (100 mg Kg^{-1}, IP). Bones –Iliac, femur and tibia- were collected, fixed in paraformaldehyde 10% and processed to be analyzed by energy dispersive X-ray fluorescence (EDXRF) technique with a limit of lead detection of 2 $\mu g\, g^{-1}$ and a threshold for lead intoxication of 5 $\mu g\, g^{-1}$ (Fig. 1)(Guimarães et al., 2012).

The experimental protocol was approved by the Ethical Committee of Faculty of Medicine, University of Lisbon and was carried out in accordance to the national and European laws on animal experimentation (63/2010/CE).

2.2. Central angiotensin antagonism

2.2.1. Anaesthesia and surgical protocol

Pb-rats were anaesthetised with sodium pentobarbitone (60 mg Kg^{-1}, IP) and artificially ventilated after neuromuscular blockade with vecuronium bromide (4 mg $Kg^{-1}\, h^{-1}$, IV). An adequate level of anaesthesia was maintained by ensuring the absence of a withdrawal reflex before the neuromuscular blockade. During this blockade, the level of anaesthesia was monitored by recording blood pressure, heart rate and central respiratory activity. The femoral artery and vein were catheterized to record blood pressure and to administer drugs and saline, respectively. The trachea was cannulated through a midline cervical incision to facilitate mechanical ventilation with O_2-enriched air using a positive pressure ventilator (Harvard Apparatus Ltd). Ventilation was regulated to maintain end-tidal CO_2 between 4.5 and 5% (ADC gas analyser, UK). Rectal temperature was maintained at 38.5 ± 0.5 °C using a servo-controlled heating blanket (Harvard Apparatus Ltd). The electrocardiogram (ECG) was recorded (Neurolog, Digitimer Ltd, UK) from needle-electrodes inserted into three of the four limbs. Heart rate was derived from the ECG (Lectromed, UK). The right carotid artery bifurcation was identified and the tip of a catheter was placed within the right carotid sinus by retrograde cannulation of the external carotid artery.

Rats were placed in a stereotaxic instrument (Kopf Instruments, Germany) such that the difference in height between lambda and bregma was zero. The activity of the left phrenic nerve was recorded with a bipolar silver hook electrode and signals were amplified and filtered (Neurolog, Digitimer). After the neuromuscular blockade, the dorsal surface of medulla was exposed by removing the atlanto-occipital membrane and a portion of the occipital bone. Microinjection was made from multibarrelled micropipettes (tip diameter, 40–60 μm) inserted into the right and left NTS using stereotaxic co-ordinates (Paxinos and Watson, 2006). The barrels of the microelectrode were filled with Wood's metal for electrical stimulation (50 Hz, 1 ms, 30 mA), sodium glutamate (2 mM, pH = 7.4 ± 0.1) and the selective angiotensin (1–7) antagonist D-Ala7-Ang-(1–7) dissolved in artificial cerebrospinal fluid (A-779, 0.5 mM, pH = 7.4 ± 0.1). The last barrel was filled with pontamine sky blue dye (2%) in sodium acetate (1 M) to mark the sites of stimulation. The NTS was functionally identified by a depressor response to injection of L-glutamate (2 mmol, Sigma). Blood pressure (BP), ECG, heart rate (HR) and phrenic nerve activity (PhrN) were monitored throughout all experiment.