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Short communication

Gestational exposure to cadmium alters crucial offspring rat brain enzyme activities: The role of cadmium-free lactation

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ARTICLE INFO

Article history:
Received 24 April 2013
Received in revised form
17 July 2013
Accepted 22 July 2013
Available online 3 August 2013

Keywords:
Cadmium
Neurotoxicity
Acetylcholinesterase
Na^+,K^+-ATPase
Mg^{2+}-ATPase
Brain

ABSTRACT

The present study aimed to shed more light on the effects of gestational (*in utero*) exposure to cadmium (Cd) on crucial brain enzyme activities of Wistar rat offspring, as well as to assess the potential protective/restorative role that a Cd-free lactation might have on these effects. In contrast to earlier findings of ours regarding the pattern of effects that adult-onset exposure to Cd has on brain AChE, Na^+,K^+- and Mg^{2+}-ATPase activities, as well as in contrast to similar experimental approaches implementing the sacrificing mode of anaesthesia, *in utero* exposure to Cd-chloride results in increased AChE and Na^+,K^+-ATPase activities in the newborn rat brain homogenates that were ameliorated through a Cd-free lactation (as assessed in the brain of 21-day-old offspring). Mg^{2+}-ATPase activity was not found to be significantly modified under the examined experimental conditions. These findings could provide the basis for a further evaluation of the herein discussed neurotoxic effects of *in utero* exposure to Cd, in a brain region-specific manner.

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1. Introduction

Cadmium (Cd) is implicated in the induction of developmental neurotoxicity and cognitive dysfunction in both humans (Schoeters et al., 2006) and animals (Faroon et al., 2012). We have already reported the effects of *in vitro* as well as *in vivo* adult-onset short- and long-term exposure to Cd on rat brain acetylcholinesterase (AChE; a crucial cholinergic enzyme) activity (Carageorgiou et al., 2004, 2005), as well as on the activities of two crucial rat brain adenosine triphosphatases (Na^+,K^+-ATPase and Mg^{2+}-ATPase; major contributors to the regulation of neuronal excitability, metabolic energy production, neurotransmission modulation and other crucial cellular functions) (Carageorgiou et al., 2004). The aim of the present study was to shed more light on the effects of gestational

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http://dx.doi.org/10.1016/j.etap.2013.07.014

(*in utero*) exposure to Cd on the above-mentioned crucial brain enzyme activities of rat offspring, as well as to assess the potential protective/restorative role that a Cd-free lactation might have on these effects.

2. Materials and methods

2.1. Animals

Eighteen albino Wistar adult female rats (two-months-old) were used and housed two in a cage, at a constant room temperature (22 ± 1 °C) under a 12 h light:12 h dark (light 08:00–20:00 h) cycle. Food and water were provided *ad libitum*. Animals were cared for in accordance with the principles for the care, use and protection of experimental animals as set by the EEC Council Directive 86/609/EEC (EEC Council, 1986) and aligned according to the Recommendation 2007/526/EU.

2.2. Mating and exposure to Cd during gestation and lactation

Nine albino Wistar adult male rats were used for mating purposes only; each male was placed with two females in each cage, in order for mating to be achieved. Following that (as assessed through the examination for the presence of an ejaculatory plug in the vagina), males were removed and female rats were equally divided into two groups: (a) controls (receiving tap water during both gestation and lactation, $n = 6$) and (b) Cd (receiving 50 ppm of Cd as Cd-chloride in the drinking water during gestation and/or lactation, $n = 12$) (Konecki et al., 2003; Sorell and Graziano, 1990). A number of newborn offspring ($n = 6$ from each group) were sacrificed at day 1, providing brain samples for the control and the $Cd_{(G)}$ (exposure to Cd during gestation) groups (Fig. 1A). The Cd-treated females were then divided into two subgroups: (a) the $Cd_{(G)}$ (exposed to Cd during gestation but not during lactation) and (b) the $Cd_{(G+L)}$ (exposed to Cd during gestation and lactation) one. At the end of the lactation period, 21-day-old rat offspring from the three groups (control, $Cd_{(G)}$ and $Cd_{(G+L)}$; $n = 6$ from each group) were sacrificed by decapitation and their brains were rapidly removed (Fig. 1A).

2.3. Tissue preparation

The brain tissue was homogenized in 10 vol ice-cold (0–4 °C) medium containing 50 mM Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl), pH 7.4 and 300 mM sucrose, using an ice-chilled glass homogenizing vessel (Potter-Elvehjem glass-teflon homogenizer) at 900 rpm (4–5 strokes). Then, the homogenate was centrifuged at 1000 × *g* for 10 min to remove nuclei and debris (Tsakiris, 2001). In the resulting supernatant, the protein content was determined according to the method of Lowry et al. (1951) and then the enzyme activities were measured.

2.4. Determination of brain AChE activity

The activity of AChE was determined by following the hydrolysis of acetylthiocholine according to the method of Ellman et al. (1961), as described by Tsakiris (2001). The incubation mixture (1 ml) contained 50 mM Tris–HCl, pH 8, 240 mM sucrose and 120 mM NaCl. The protein concentration of the incubation mixture was 80–100 μg/ml. The reaction was initiated after addition of 0.03 ml of 5,5′-dithionitrobenzoic acid (DTNB) and 0.05 ml of acetylthiocholine iodide, which was used as substrate. The final concentration of DTNB and substrate were 0.125 and 0.5 mM, respectively. The reaction was followed spectrophotometrically with a Hitachi U-1800 UV–vis spectrophotometer (Japan) by the increase of absorbance $\Delta\overline{OD}$ at 412 nm.

2.5. Determination of Na^+,K^+-ATPase and Mg^{2+}-ATPase activities

(Na^+,K^+)-ATPase activity was calculated from the difference between total ATPase activity (Na^+,K^+,Mg^{2+}-dependent ATPase) and Mg^{2+}-dependent ATPase activity. Total ATPase activity was assayed in an incubation medium consisting of 50 mM Tris–HCl, pH 7.4, 120 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 240 mM sucrose, 1 mM ethylenediamine tetraacetic acid K_2-salt (K^+-EDTA), 3 mM disodium ATP and 80–100 μg protein of the homogenate in a final volume of 1 ml. Ouabain (1 mM) was added in order to determine the activity of Mg^{2+}-ATPase. The reaction was started by adding ATP and stopped after an incubation period of 20 min by addition of 2 ml mixture of 1% lubrol and 1% ammonium molybdate in 0.9 M H_2SO_4 (Bowler and Tirri, 1974; Tsakiris, 2001). The yellow colour which developed was read at 390 nm.

2.6. Chemicals

All chemicals used in this study were of analytical grade and/or of the highest purity available and were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.7. Statistical analysis

The data were analyzed using Student's *t*-test. All analyses were performed by SPSS for Windows Software. Values of $p < 0.05$ were considered statistically significant.

3. Results

Newborn rats exposed to Cd during gestation demonstrated a statistically-significant increase in their brain AChE (+29%, $p < 0.001$; Fig. 1B) and Na^+,K^+-ATPase (+30%, $p < 0.01$; Fig. 1C) activities (as compared to controls). Contrary to these findings, exposure to Cd during both gestation and lactation (group $Cd_{(G+L)}$) resulted in a statistically-significant decrease in AChE activity (−11%, $p < 0.05$; Fig. 1B) as compared to the control group, while it had no significant effect on Na^+,K^+-ATPase activity (Fig. 1C). Both enzyme activities returned to control levels in the $Cd_{(G)}$ group due to Cd-free lactation (Fig. 1B and 1C). Moreover, no significant changes were observed with regards to Mg^{2+}-ATPase activity in either newborn or 21-day-old offspring brains, following exposure to Cd (Fig. 1D).

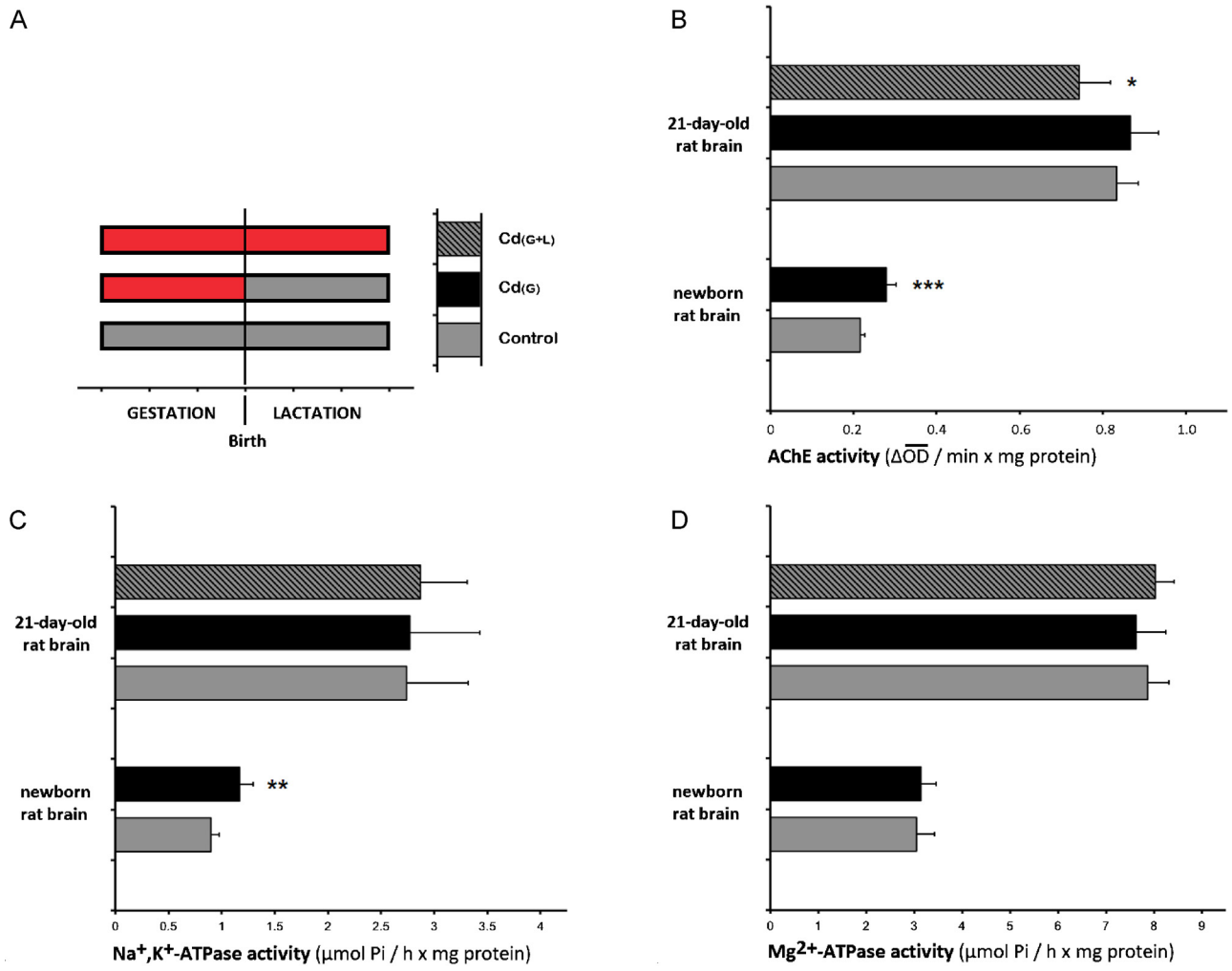

Fig. 1 – Effects of gestational ($Cd_{(G)}$) or gestational and lactational ($Cd_{(G+L)}$) exposure to cadmium (Cd; schematic timescale of exposure highlighted with red colour) (A), on offspring rat brain acetylcholinesterase (AChE) (B), Na^+,K^+-ATPase (C) and Mg^{2+}-ATPase (D) activities. Each value in (B)–(D) indicates the mean ± SD of six independent experiments. The average value of each experiment was obtained from three evaluations in the homogenized rat brain of newborn (1-day-old) or 21-day-old rat offspring. $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

The activity of AChE is a neurochemical parameter that should be discussed with caution when it comes to its modulation by Cd and/or other neurohepatotoxic environmental pollutants (Zarros et al., 2012). We have recently reviewed the effects of Cd on the brain AChE activity of various species, as reported through *in vivo* and *in vitro* experimental approaches (Zarros et al., 2013), and have underlined the importance of various experimental issues affecting the validity of this parameter as a biomarker. Antonio et al. (2003) have reported that exposure of Wistar rat dams to a solution of Cd-acetate (10 mg/l in the drinking water) throughout gestation and lactation resulted in a non-significantly altered AChE activity in the Cd-exposed newborn (day 0) rat brain, but significantly inhibited this crucial cholinergic enzyme in the 21-day-old offspring rat brain. Moreover, it has been recently reported that exposure of Sprague-Dawley rat dams to Cd under the same experimental conditions (solution of 10 mg/l Cd-acetate in the drinking water throughout gestation and lactation) resulted in a non-significantly altered AChE activity in either the Cd-exposed newborn (day 0) or 21-day-old offspring rat brains (Zhang et al., 2009). The herein reported Cd-induced inhibition of AChE in the 21-day-old offspring rat brain (Fig. 1B) is in agreement with the above references (Antonio et al., 2003; Zhang et al., 2009), while a possible reason for the novelty of the observed increased AChE activity in the newborn rat brain is the use of anaesthesia (ether) by the above authors (Antonio et al., 2003; Zhang et al., 2009) as a means of sacrifice (Zarros et al., 2013), in contrast to our sole use of decapitation. It should be noted that the use of anaesthesia might exert a prolonged inhibitory effect on brain AChE activity in rats (Vernadakis and Rutledge, 1973) previously exposed to neurohepatotoxicants (such as

Cd or manganese), and thus might generate artifacts when compared to non-exposed controls (this argument has been recently discussed by Zarros et al., 2012, 2013). Interestingly, our data from adult-onset Cd-induced neurotoxicity experiments have demonstrated a decreased rat brain AChE activity following a short-term exposure (Carageorgiou et al., 2004) and an increased rat brain AChE activity following a long-term exposure (Carageorgiou et al., 2004, 2005). These data underline the existence of a totally different reaction of this neurochemical parameter within the developing rat brain.

Gupta et al. (1991) have reported that exposure of Drukery rat dams to 50 ppm of Cd (as Cd-acetate in the drinking water) throughout gestation did not significantly alter the brain AChE or Na^+,K^+-ATPase in the Cd-exposed 7- and 14-day-old offspring, but caused a significant inhibition of both enzymes in the 21-day-old offspring rat brain. None of these findings are in agreement with ours, as Cd-free lactation restored both Wistar rat brain enzyme activities to control levels (Fig. 1B and 1C). Antonio et al. (2003) have also reported a decreased Na^+,K^+- and Ca^{2+}/Mg^{2+}-ATPase activity in the brains of 21-day-old Wistar rat pups exposed to Cd throughout gestation and lactation. Moreover, in another interesting study, Rajanna et al. (1990) have evaluated the neurotoxic effects of chronic exposure to low levels of Cd (5 and 10 μg/l in the drinking water) from birth through 4 months and have reported a significant decrease in the activities of brain synaptosomal Na^+,K^+-ATPase and oligomycin-sensitive Mg^{2+}-ATPase in parallel with a decrease in [^{3}H]-dopamine and [^{3}H]-norepinephrine uptake and an increase in lipid peroxidation. Their findings, however, could not easily be correlated with ours, as the experimental procedure and the timeframes examined do not match.

In conclusion, our study has revealed that in contrast to earlier findings of ours (Carageorgiou et al., 2004, 2005) regarding the pattern of effects that adult-onset exposure to Cd has on brain AChE, Na^+,K^+- and Mg^{2+}-ATPase activities, as well as in contrast to similar experimental approaches implementing the sacrificing mode of anaesthesia (Antonio et al., 2003; Zhang et al., 2009), *in utero* exposure to Cd-chloride results in increased AChE and Na^+,K^+-ATPase activities in the newborn rat brain homogenates that can be ameliorated through a Cd-free lactation. Continuous exposure to Cd through both gestation and lactation only seems to affect rat brain AChE activity, by inhibiting it. Interestingly, Mg^{2+}-ATPase activity was not found to be significantly modified under the examined experimental conditions (Fig. 1D). Our study could provide the basis for a further evaluation of the herein discussed neurotoxic effects of *in utero* exposure to Cd, in a brain region-specific manner.

Conflict of interest statement

Nothing declared.

Acknowledgments

This study was funded by the National and Kapodistrian University of Athens. The authors wish to acknowledge their appreciation to Ms. Vasiliki Gkanti for her assistance.

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