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Influence of prenatal exposure to environmental pollutants on human cord blood levels of glutamate

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ABSTRACT

Some chemicals released into the environment, including mercury and some organochlorine compounds (OCs), are suspected to have a key role on subclinical brain dysfunction in childhood. Alteration of the glutamatergic system may be one mechanistic pathway. We aimed to determine whether mercury and seven OCs, including PCBs 138, 153, and 180, DDT and DDE, hexachlorobenzene (HCB), and beta-hexachlorocyclohexane (β-HCH) influence the cord levels of two excitatory amino acids, glutamate and aspartate. Second, we evaluated if this association was mediated by glutamate uptake measured in human placental membranes. The study sample included 40 newborns from a Spanish cohort selected according to cord mercury levels. We determined the content of both amino acids in cord blood samples by means of HPLC and assessed their associations with the contaminants using linear regression analyses, and the effect of the contaminants on glutamate uptake by means of [^{3}H]-aspartate binding in human placenta samples. PCB138, β-HCH, and the sum of the three PCBs and seven OCs showed a significant negative association with glutamate levels (decrease of 51, 24, 56 and 54%, respectively, in glutamate levels for each 10-fold increase in the contaminant concentration). Mercury did not show a significant correlation neither with glutamate nor aspartate levels in cord blood, however a compensatory effect between T-Hg and both PCB138, and 4,4′-DDE was observed. The organo-metallic derivative methylmercury completely inhibited glutamate uptake in placenta while PCB138 and β-HCH partially inhibited it (IC_{50} values: 4.9 ± 0.8 μM, 14.2 ± 1.2 nM and 6.9 ± 2.9 nM, respectively). We conclude that some environmental toxicants may alter the glutamate content in the umbilical cord blood, which might underlie alterations in human development.

1. Introduction

Social and economic development have been mediated by strong industrial growth involving the synthesis, use and release of large numbers of chemicals many of which are potentially harmful to human health and the environment (Li and Macdonald, 2005; Porta et al., 2012). Several of these chemicals, e.g. lead, mercury, polychlorinated biphenyls (PCBs), arsenic and toluene, have been associated to subclinical brain dysfunction and neurodevelopmental disorders such as poorer cognitive and motor development, attention deficit, hyperactivity, and sensory deficits but the underlying processes leading to these effects are still unknown (Grandjean and Landrigan, 2006). Pollutants come from several different sources, move on the environment and eventually accumulate in the food chain. Daily exposure to environmental persistent pollutants results in detectable levels of metals, PCBs and organochlorine pesticides in woman bodies (Woodruff et al., 2011). During pregnancy, the pollutants accumulated by the women can reach the fetus crossing the placenta. Although the placenta regulates the exchange of nutrients and oxygen between mother and fetus and acts as a barrier of many toxicants, it does not

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provide protection against certain environmental contaminants (Prouillac and Lecoeur, 2010).

The glutamatergic system is one of the targets of several pollutants in the nervous system (Allen et al., 2001; Briz et al., 2010; Fonfría et al., 2005; Fonnum and Lock, 2004; Hogberg et al., 2010; Piedrafita et al., 2008). Glutamate is a non-essential amino acid that has a key role in metabolic pathways, being important for the normal growth and development of the fetus during pregnancy. Glutamate is produced by the fetal liver using maternal glutamine, and the excess of glutamate from the fetal circuit is removed by glutamate transporters (namely excitatory amino acid transporters: EAAT1, EAAT2 and EAAT3) in placenta (Battaglia, 2000; Noorlander et al., 2004). Glutamate is also the major excitatory neurotransmitter in the central nervous system of mammals and it is present in human fetal brains at concentrations that did not vary during the third trimester of pregnancy (Girard et al., 2006). It mediates key aspects of normal brain function such as sensory information, motor coordination, emotions and cognition. However, it has neurotoxic effects when it is present in excess (Danbolt, 2001). On the other hand, the amino acid aspartate shares the same uptake transport than glutamate. Although its role as a neurotransmitter has not yet been established, recent studies suggest that it may play neuromodulatory functions at developmental stages by acting on glutamate receptors (Errico et al., 2012; Ota et al., 2012).

Mercury has been reported to inhibit glutamate transport in neural cells and to increase glutamate release into the extracellular space of neural cells and platelets (Allen et al., 2001; Borges et al., 2007; Fonfría et al., 2005). Specific attention should be paid to its organometallic compound, methylmercury, because: (i) it has recognized properties as a human neurodevelopmental toxicant (Grandjean and Landrigan, 2006); (ii) poisoning incidents with methylmercury have demonstrated the potential of this pollutant as neurotoxicant and its serious health consequences (Castoldi et al., 2008; Ekino et al., 2007; Grandjean et al., 2010; Nakagawa et al., 2002); (iii) millions of people are nowadays chronically exposed to this contaminant and there is epidemiological (Marsh et al., 1987; Pinheiro et al., 2007; Yokoo et al., 2003) and experimental (Castoldi et al., 2008; Farina et al., 2009; Vendrell et al., 2007, 2010) evidence of the toxic effects of chronic exposure to this metal especially for the nervous system; and (iv) previous results from the INMA birth cohort of Valencia (Spain) showed elevated levels of this pollutant in umbilical cord blood, where 70% of newborns had mercury levels above US EPA recommended level, i.e. 5.8 μg/L of MeHg (Ramon et al., 2008).

Moreover, organochlorine compounds (OCs) such as PCBs and dieldrin have also been described to alter glutamate neurotransmission (Briz et al., 2010; Llansola et al., 2010; Mariussen and Fonnum, 2001; Piedrafita et al., 2008; Stavenes Andersen et al., 2009), which may lead to neurotoxicity. Prenatal exposure to some of them are of high concern since levels above the limit of quantification have been found in a significant percentage of samples of several mother-child cohorts including the INMA cohort of Valencia (Vizcaino et al., 2010, 2011). Furthermore, neuropsychological studies on children reveal an association between PCB prenatal exposure and impaired neuropsychological and psychomotor development (Cheslack-Postava et al., 2013; Fernández et al., 2012; Forns et al., 2012a,b; Sagiv et al., 2012; Gascon et al., 2013). It is also important to consider that people are simultaneously exposed to several pollutants through the diet (Arrebola et al., 2011; Llop et al., 2010; Ramon et al., 2008) so the effects induced by the pollutants mixture may be different than the ones observed in experimental works with isolated pollutants.

The aim of the present study is to address whether mercury and some OCs alter glutamate uptake in human placental membranes and, thus, influence the levels of excitatory amino acids in cord blood. For this purpose, in the frame of a mother and child cohort study (INMA: Infancia y Medio Ambiente/Childhood and Environment; www.proyectoinma.org) 40 cord blood samples with known concentrations of mercury and seven organochlorines, including PCBs, DDTs, hexachlorobenzene (HCB), and beta-hexachlorocyclohexane (β-HCH) have been analyzed for their content in two excitatory amino acids, glutamate and aspartate. The associations between these amino acids and the OCs and mercury have been analyzed. Binding assays with human placental homogenates exposed to methylmercury and OCs have also been performed.

2. Material and methods

2.1. Population and sample collection

2.1.1. Population

Informed consent was obtained from all participants and the study was approved by the Hospital La Fe (Valencia, Spain) Ethics Committee. The study population included 40 mother-child pairs among the 787 deliveries of the INMA-Valencia cohort (Guxens et al., 2012). Selection criteria of the samples were based on availability of cord blood and placenta samples for the determinations as well as on total mercury (T-Hg) concentrations. Thus, the consecutive 20 children with the highest levels of exposure to T-Hg and the successive 20 with the lowest levels were selected. All children in the highest group had levels of mercury >20 μg/L and, in the case of the group of lower levels, all children had <6.6 μg/L of mercury. The selected samples also had cord blood analyses of some OCs, including HCB, β-HCH, 4,4′-DDT, 4,4′-DDE, and PCBs 138, 153 and 180. Total cholesterol and triglycerides were determined by means of enzymatic techniques, calculating total serum lipid concentrations (Phillips et al., 1989).The pollutant levels of the INMA-Valencia cohort, the laboratory analytical methods and quality control procedures are described elsewhere (Lopez-Espinosa et al., 2010; Ramon et al., 2008, 2011; Vizcaino et al., 2010, 2011). Levels of these contaminants (n = 40) have been described in Table 1.

2.1.2. Umbilical cord and placenta samples

Umbilical vein blood samples were collected using venipuncture of cord vessels before the placenta was delivered. Serum and plasma samples were obtained after whole blood centrifugation at 2500 rpm and separated into aliquots of 1 mL. Placentas collected at delivery were examined, and pieces of maternal and fetal sides were immediately dissected. Both cord and placenta samples were coded, frozen and stored confidentially and anonymously at −80 °C until processed.

2.2. Chemicals

Sacarose, Tris-HEPES, glacial acetic acid and protease inhibitor cocktail (P8340) were purchased from Sigma (St. Louis, USA). Chemical standards, methylmercuric chloride, PCB 138 and β-HCH were from ICN (Cleveland, OH, USA), Dr. Ehrenstoner GmbH (Augsburg, Germany), and National Physical Laboratories UK (Teddington, United Kingdom), respectively. [^{3}H]-D-aspartate (27 Ci/mmol) was from Amersham Life Sciences (Buckinghamshire, UK). Optiphase 'Hisafe' 2 liquid scintillation cocktail was from Wallace Oy (Turku, Finland). The chemicals were dissolved and diluted in Tris-acetate buffered solution or in dimethyl sulfoxide solution (DMSO). When dissolved in DMSO, a 200× concentration was prepared, thus the concentration of DMSO in the testing solution was 0.5%. Controls contained the same amount of DMSO, when so required.

L-Glutamic acid, L-aspartic and *o*-phthalaldehyde/-mercaptoethanol (50:50) reagent solutions were from Sigma (St. Louis, USA).