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Characterization of placental cholinesterases and activity induction associated to environmental organophosphate exposure

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ABSTRACT

Although non-innervated, the placenta contains both cholinesterases (ChEs), butyrylcholinesterase (BChE) and acetylcholinesterase (AChE). These enzymes are well-known for their multiple molecular forms. In a first approach, we used recognized specific inhibitors, substrate preferences and non-denaturating gel electrophoresis in order to characterize the ChE profile of term placenta from uncomplicated pregnancy. Results strongly suggest that the predominant cholinesterasic form present was tetrameric BChE.

It is well established that both ChEs are targets of cholinesterase-inhibiting organophosphates (OP), one of the most important classes of chemicals actively applied to the environment. However, we have previously reported increased ChEs activity in placenta of rural residents exposed to OP. In the present work, we have studied: 1) whether this finding was reproducible and, 2) whether AChE or BChE up regulation is behind the increase of placental ChE activity. The population studied included forty healthy women who live in an agricultural area. Samples were collected during both the OP pulverization period (PP) and the recess period (RP). The placental ChEs activity increased in PP, evidencing reproducibility of previous results. The analysis of non-denaturating gels revealed that increased activity of total ChE activity in placenta from women exposed to OP may be attributable to tetrameric BChE up-regulation.

1. Introduction

Humans have two cholinesterases (ChEs): acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). The enzymatic functions of both enzymes include hydrolysis of acetylcholine ACh [1]. At the nerve synapses, AChE terminates nerve impulse transmission by hydrolyzing this neurotransmitter. On the other hand, BChE acts as a backup for AChE and as a scavenger for poisons that might inhibit AChE activity [2]. These enzymes have been very rapidly distinguished and subject of considerable research [3].

Abbreviations: ChEs, cholinesterases; BChE, butyrylcholinesterase; AChE, acetylcholinesterase; ChE, cholinesterase; OP, organophosphates; PP, pulverization period; RP, recess period; ACh, acetylcholine; ASCh, acetylthiocholine iodide; BSCh, butyrylthiocholine iodide; iso-OMPA, tetraisopropylpyrophosphoramide; BW284C51, 1,5-bis (4-allyldimethyl ammoniumphenyl)-pentan-3-one dibromide.

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AChE and BChE are well-known for their multiple molecular forms [4]. Polymorphism is achieved by certain combinations of alternative gene splicing, and by the attachment of non-catalytic structural subunits. In mammals, AChE is encoded by a single gene. However, alternative splicing at the C-terminus of AChE mRNA generates three different isoforms. Conversely, one BChE transcript has been identified so far [5].

The presence of ChEs in tissues that are not cholinergically innervated provides the most compelling evidence that both AChE and BChE might have functions, other than the termination of cholinergic neurotransmission [6]. In fact, the human placenta contains an active cholinergic system which was associated to the amino acid uptake, the release of human placental chorionic somatotropin and prostaglandin production [7] and to the modulation of nitric oxide effect [8].

The concentrations of AChE and BChE are considerably lower in the placenta than in the nervous system [9]. The analysis by electron microscopy of cross sections from term placenta, cytochemically stained for ChEs activities, showed that term placenta syncytiotrophoblast cells produce primarily AChE. On the other hand, epithelial cells that surround the inner part of blood vessels, as well as hematopoietic cells present in them, all intensely stained for both AChE and BChE activities [10]. In accordance with these observations, it was reported that both AChE and BChE activities were detectable in cultured explanted villous of term placenta [11].

Depending on the experimental conditions used, dissimilar OP effects on placental AChE activity have been reported. Gestational exposure of rats to oral doses of the OP chlorpyrifos cause no inhibition of AChE activity [12], while a single cutaneous dose of OP in pregnant rats decreased AChE activity [13]. Nevertheless, we previously reported increased ChE activity in human placenta associated to OP environmental exposure [14].

Considering that AChE up regulation was induced post OP-treatment in rodents brain [3,15], muscle [16] and plasma [17], we speculate that an adaptive change could explain our previous finding.

The objective of the present study was to verify the reproducibility of the increased placental ChE activity associated to OP environmental exposure and to determine whether AChE up regulation is behind this finding. In addition, we also characterized placental ChEs activity in control samples using recognized specific inhibitors.

2. Materials and methods

2.1. Chemicals

Acetylthiocholine (ASCh) iodide, butyrylthiocholine (BSCh) iodide, 5,5′-dithio-bis (c-nitrobenzoic acid) (DTNB), eserine hemisulfate salt, tetraisopropylpyrophosphoramide (iso-OMPA), 1,5-bis (4-allyldimethyl ammonium-phenyl)-pentan-3-one dibromide (BW284C51), HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), EDTA (etilen diamino-tetracetic acid), boric acid, bovine serum albumin, Tris, glycerol, bromophenol blue, maleic acid, sodium citrate dihydrate, copper pentahydrate, potassium ferricyanide, cholinesterase acetyl (True Cholinesterase EC 3.1.1.7) Type V-S from Electric Eel, acrylamide, tetramethylenediamine and ammonium persulfate were purchased from SIGMA. Sodium dodecyl sulphate (99% pure) and ethanol (99% pure) were purchased from MERCK (Germany). Bisacrylamide was acquired from PROMEGA.

2.2. Participant recruitment and collection of samples

We performed a study of 40 healthy women ranging between 15 and 36 years of age incoming to prenatal care at the Cinco Saltos Public Hospital (Río Negro Province, Argentina), between December 2006 and August 2008. They were asked by a physician to participate in the study during their third trimester of pregnancy and, informed consent was obtained from each participant before they were interviewed. This study was carried out with the full ethical approval of the local Advisory Committee of Biomedical Research in Humans. The patients included in this study were residents of farms or communities surrounding fruit cultivation areas where pesticides, such as the OPs azinphos methyl, phosmet, chlorpyrifos and dimethoate, are applied during the spring and summer (September to February). Pesticides are usually finely dispersed as droplets at the time of pulverization and aerial drift from the target area is frequently, increasing the potential environmental exposure of the population. Samples collected from September to December were considered samples from the PP, and those collected from April to August were considered samples from the non-pulverization period or recess period (RP). A questionnaire was administered to document physical characteristics, educational level and lifestyle habits. Women with chronic diseases, on long-term medication (except those included in Group A according to the FDA), and those with serious pregnancy complications were excluded. Groups were matched for reported smoking habit and alcohol consumption. Placental villous samples were collected within 20 min of vaginal delivery. Suitable amounts from the central area of the maternal side of the placenta were obtained as the expression of various components that might vary according to the location. Samples were frozen at −20 °C until use.

In addition, placentas from urban residents with no history of pesticide exposure were collected during July–August 2006 to characterize placental ChEs activity. Similar exclusion criteria as those of the population study were used. Also, the full the local Advisory Committee of Biomedical Research in Humans approved this part of the study.

2.3. Cholinesterases activity and characterization

Small pieces of the tissue were cut and repeatedly washed with physiological solution and homogenized in ice-cold buffer. Then homogenates were filtered through a muslin cloth and centrifuged at 4 °C during 5 min at 4000 × *g*. AChE and BChE activities were determined in the supernatant according to the method of Ellman et al. [18]. In a typical assay, 2.6 ml of 0.1 M phosphate buffer pH 8, 100 μl of 0.01 M DTNB and 400 μl of the