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Original article

Cerebellar level of neurotransmitters in rats exposed to paracetamol during development

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ABSTRACT

Background: The present study was designed to clarify the effect of prenatal and postnatal paracetamol administration on the neurotransmitter level and balance of amino acids in the cerebellum.
Methods: Biochemical analysis to determine the concentration of neurotransmitters in this brain structure was performed on two-month-old Wistar male rats previously exposed to paracetamol in doses of 5 (P5, $n = 10$) or 15 mg/kg (P15, $n = 10$) throughout the entire prenatal period, lactation and until the completion of the second month of life, when the experiment was terminated. Control animals were given tapped water (Con, $n = 10$). The cerebellar concentration of monoamines, their metabolites and amino acids were assayed using High Performance Liquid Chromatography (HPLC).
Results: The present experiment demonstrates that prenatal and postnatal paracetamol exposure results in modulation of cerebellar neurotransmission with changes concerning mainly 5-HIAA and MHPG levels.
Conclusion: The effect of paracetamol on monoaminergic neurotransmission in the cerebellum is reflected by changes in the level of catabolic end-products of serotonin (5-HIAA) and noradrenaline (MHPG) degradation. Further work is required to define the mechanism of action and impact of prenatal and postnatal exposure to paracetamol in the cerebellum and other structures of the central nervous system (CNS).

Introduction

Paracetamol (acetaminophen, *N*-acetyl-*p*-aminophenol) is at the top of the list of over-the-counter medications taken prenatally. The drug passes freely through the placenta and can affect fetal development, but most clinical trials have not confirmed the association between exposure to paracetamol in the first trimester of pregnancy and an increased risk of congenital abnormalities [1–4].

Although the drug has been used for many years as a first-choice analgesic and antipyretic during pregnancy and in infancy, its effect on the early neural development of the central nervous system (CNS) has not been sufficiently explored. In particular, the impact of prenatal and early postnatal exposure on cerebellar development and function is little known. In the course of ontogenesis, cerebellar structures develop relatively early and from the beginning operate in close connection with the motor centers in the CNS. The cerebellar neurons arise in a sequential order: deep nuclei neurons in charge of output are generated first, interneurons are produced later [5,6]. Meanwhile, the cerebellum is responsible for maintaining body balance, control of eye movements and participation in the planning, initiating and proper coordination of movements. This part of the brain allows to adjust incorrect movements, as well as learn and memorize the new motor skills and their automation.

Paracetamol has a multidirectional effect on the CNS and displays a complex mechanism of action, which definitely makes it hard to speculate on possible long-term consequences of the drug on neural development and cognition. Its analgesic and antipyretic properties are probably associated with inhibition of the centrally situated isoform of the cyclooxygenase enzyme COX-3 and suppression of elementary prostaglandin synthesis. Other

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aspects of central actions are also identified, e.g. stimulation of transient receptor potential cation channel A1 (TRPA1) protein by the active metabolites of paracetamol, interaction with opioid receptors and with serotonergic, noradrenergic and cholinergic systems [7–10].

Even though human studies indicate paracetamol innocuousness, further neurotoxicological and behavioral studies are needed to demonstrate the harmlessness of paracetamol administration in the prenatal and early postnatal periods. In general, in infants the metabolic clearance and elimination capacity of paracetamol is below the expected values and for full safety use of the drug should be limited to 48–72 h [8–14].

An alarming cohort clinical study concerning the effects of early exposure to paracetamol on motor skills and the risks of adverse psychomotor and behavioral outcomes confirm a possible connection between prenatal drug exposure and neurodevelopmental disorders [15]. At the same time, it has been shown that early postnatal exposure to COX inhibitors alters cerebellar Purkinje cell development [16]. In rats paracetamol affects dendritic length and lead to sex-dependent changes in the cerebellum, e.g. a decrease of dendritic spines, cell atrophy and loss of cerebellar volume in males. This indicates subtle sex-based differences in cerebellar responses. The cerebellum integrates sensory information and cognitive tasks such as working memory [17]. In the present study, we have decided to see how paracetamol administered prenatally and in the early ontogenesis affects the cerebellar neurotransmission.

Materials and methods

Animals and paracetamol treatment

Virgin, three-month old Wistar (Albino Glaxo) rat females were mated and the day when the vaginal plug was detected was considered the first gestation day. Administration of paracetamol began with the first day of pregnancy. Pregnant females ($n = 15$) were randomly assigned to paracetamol treated groups or control group and placed individually in plastic breeding cages in a room with controlled temperature (24–26 °C) and 12 h dark-light cycle. During pregnancy and lactation mothers were given free access to standard food (Labofeed, Kcynia, Poland) and tap water or solution of paracetamol. Drugs solutions were administered orally in drinking water, and concentrations were adjusted to an average daily intake of 5 (group P5) and 15 mg/kg (group P15) per rat. In order to minimize dosing error, we have controlled the quantity of a drinking drug solution each day to properly adjust the medication dose.

The dams were checked daily in the morning for pups. After birth, male offspring was left with dams until 28 days of age. After weaning five animals per cage were placed and oral administration of paracetamol at doses of 5 (P5, $n = 10$) and 15 mg/kg (P15, $n = 10$) was continued for the second month of life. Control pups received water (Con, $n = 10$). The effect of maternal and early life exposure to low doses of paracetamol was assessed in two-month old male rats. All procedures were carried out in compliance with the ethical standards of the Directive 2010/63EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Biochemical assessment

The cerebellar concentrations of monoamines, their metabolites and amino acids were estimated in 30 rats. Control and treated rats were sacrificed at two months of age. Rats offspring were decapitated and samples for evaluation of cerebellum neurotransmitters by high-performance liquid chromatography (HPLC) were immediately sectioned on ice. Cerebellar tissue was rapidly weighed, flash frozen and stored in a deep freezer (−80 °C) for future biochemical analysis. To precipitate proteins, tissues were homogenised in ice-cold 0.1 N perchloric acid and centrifuged at 13,000 × g for 15 min at 4 °C. The supernatant was collected, filtered (0.2 μm pore size filter; Whatman) and a 20 μL aliquot was injected onto the HPLC.

The concentration of cerebellar dopamine (DA) and corresponding metabolites: 3,4-dihydroxyphenyl acetic acid (DOPAC), homovanillic (HVA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), noradrenalin (NA) and 3-methoxy-4-hydroxyphenylglycol (MHPG) was determinated using a HPLC procedure, as previously described by our group [18]. In summary, L-3500 A detector (Merck) with electrochemical detection and glassy carbon electrode was employed, the electrochemical potential was set at 0.8 V, the mobile phase comprised 32 mM sodium phosphate, 34 mM citric acid, 1 mM octane sulfonic acid and 54 μM ethylenediaminetetraacetic acid in deionised water containing 12% methanol. Monoamines were separated on a C-18 column (Nucleosil, Macherey-Nagel, Germany) and the mobile phase flow rate was maintained at 0.8 ml/min. Data were collected and analysed by Eurochrom 2000 for Windows (Knauer, Germany). Contents of neurotransmitters and metabolites were expressed as ng/g of tissue.

The protocol for assessment of concentrations of amino acids neurotransmitters; glutamic acid (GLU), aspartate (ASP), alanine (ALA), histidine (HIS), γ-aminobutyric acid (GABA) and taurine (TAU), followed the previously described procedure [18]. The final amount of amino acids in the tissue sample were expressed as ng/mg brain tissue. All standards were purchased from Sigma-Aldrich (St. Louis, USA).

Statistical analysis

Comparison of monoamines, metabolites and amino acids concentration between-groups was performed by using one-way analysis of variance ANOVA. Significant effects were examined subsequently by Newman-Keuls test (NK) to determine specific differences. All data are presented as mean ± SE. The accepted level of significance for all tests was $p < 0.05$. All results without statistical significance with p above this value were marked as not significant (N.S.).

Results

Cerebellar monoamines concentration

The level of monoamines and their metabolites in the cerebellum are summarised in Table 1.

NA and metabolites

ANOVA did not demonstrate any statistically significant difference between the content of NA in the cerebellum between all experimental groups ($F_{(2,27)} = 1.61$, N.S.). Statistically significant differences between groups in the cerebellar MHPG concentration were observed ($F_{(2,27)} = 3.49$, $p < 0.05$). The P15 group had significantly higher level of MHPG compared with that in the control group ($p < 0.05$, NK) (Fig. 1). The ANOVA demonstrated statistically significant differences between groups in the MHPG/NA ratio in the cerebellum ($F_{(2,27)} = 4.14$, $p < 0.05$). Analysis of differences between groups showed that cerebellar MHPG/NA ratio was significantly higher in the P15 group compared to the P5 group ($p < 0.05$, NK). There were no significant differences between the control and any of the paracetamol treated groups in the MHPG/NA ratio.