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Early paracetamol exposure decreases brain-derived neurotrophic factor (BDNF) in striatum and affects social behaviour and exploration in rats

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ABSTRACT

The biochemical and behavioral responses to prenatal and early postnatal exposure to paracetamol in rats are not well understood. The effect of daily maternal and early life administration of 5 mg/kg (group P5) or 15 mg/kg paracetamol (group P15) was evaluated in two-month old male rats, relative to control animals receiving tap water (Con). Social behavior and episodic memory were investigated with Social Interaction and Novel Object Recognition (NOR) tests. Quantification of brain-derived neurotrophic factor (BDNF) was determined in prefrontal cortex, hippocampus and striatum using enzyme-linked immunosorbent assay (ELISA).

Control animals exhibited a higher total frequency of social interactions and greater frequency of sniffing compared to rats exposed to paracetamol, and we found a statistically significant increase in the occurrence of pinning in paracetamol-treated animals. Rats from the 15 mg/kg group exhibited a greater interest in objects in the NOR test and spent more time exploring objects during the familiarization and choice phases. Biochemical analysis showed significant differences in striatal BDNF between the groups, specifically, a nearly two-fold decrease in striatal BDNF in the paracetamol groups (P5: 6.78 ± 0.60 pg/mg; P15: 6.06 ± 0.46 pg/mg) relative to the control group (Con: 11.33 ± 2.00 pg/mg). These results indicate that paracetamol exposure induces changes in social behaviour and exploration in rats and results in a significant decrease of striatal BDNF.

1. Introduction

Paracetamol remains the most common over the counter drug used in pregnancy due to an absence of alternative drugs with equally favorable safety profiles. However, the long term effects of early fetal exposure to the drug are unknown.

We hypothesize that early exposure to paracetamol can cause negative effect on offspring's behaviour by inducing changes in concentration of neurotrophic factors such as BDNF.

Most studies do not provide strong support for a change in clinical practice regarding the use of paracetamol by pregnant women, despite reports of relationships between paracetamol intake and increased risks of preterm birth in mothers with pre-eclampsia, congenital abnormalities, and attention-deficit/hyperactivity disorder or hyperkinetic disorders in children (Liew et al., 2014; Rebordosa et al., 2008; Rebordosa et al., 2009; Thompson et al., 2014). Recently published results from population-representative cohort studies indicate that prenatal paracetamol exposure can adversely affect later neurodevelopmental outcomes, and the occurrence and severity of hyperactivity, impulsivity, and attention deficit symptoms has been shown to vary by frequency of drug exposure and gender (Avella-Garcia et al., 2016). Authors link the prevalence of behavioral problems with the early use of paracetamol and suggest that paracetamol exposure may be a potential contributor to the development of attention-deficit/hyperactivity disorder (ADHD) and autism (Avella-Garcia et al., 2016; Schultz and Gould, 2016).

The potential impacts of paracetamol on memory and social behavior are not well understood. A social science dissertation presented by Mischkowsky (2015) describes the social and psychological side effects of paracetamol in humans and suggests that pharmacologically ameliorated responsiveness to physical pain may also reduce empathy and therefore the cognitive, affective, and behavioral responsiveness to the pain of others.

Doubts about the safety of exposure to this drug during the prenatal and early postnatal periods motivated this study assessing the cognitive and social behavior of rats exposed to paracetamol. We additionally examine brain-derived neurotrophic factor (BDNF) levels in the prefrontal cortex, hippocampus, and striatum. The role of BDNF in the modulation of early neuronal development is well established. This

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protein plays a key role in embryogenesis by enhancing the differentiation and survival of a variety of neuronal populations in the central nervous system, regulates the activity of brain monoaminergic systems, and drives synaptic plasticity (Siuciak et al., 1996; Vaynman et al., 2004). BDNF dysfunctions have been implicated in the pathophysiology of various psychiatric and neurological disorders including depression, suicidal behaviour, schizophrenia, dementia, Rett syndrome, and Alzheimer's disease (Borba et al., 2016; Li and Pozzo-Miller, 2014; Sher, 2011; Siuda et al., 2017). Changes in the sensitivity and function of hippocampal 5-HT_{1A} and 5-$HT_{2A/C}$ receptors are closely correlated with enhanced BDNF production and can generate antidepressant and anxiolytic activity (Bambico et al., 2016). Altered BDNF/TrkB signaling in the hippocampus leads to behavioral deficits observed as increased immobility in the tail suspension test and reduced social recognition (Guida et al., 2017), as well as morphological rearrangements of non-neuronal cells in brain areas controlling emotional behavior.

Here we investigate the consequences of maternal paracetamol use on the cognitive, motor, and social development of the offspring.

2. Material and methods

2.1. Animals and paracetamol treatment

Rat pups born from dams receiving a solution of either 5 mg/kg (P5) or 15 mg/kg body weight (b.w.) (P15) paracetamol in drinking water were included in this study. The control group received tap water (Con). Three-month old Wistar (Albino Glaxo) females were mated overnight with males of the same stock, and the first day of pregnancy was assessed by the presence of vaginal plug or sperm in the vaginal smear. Pregnant females were randomly assigned to the experimental groups (Con, P5, or P15) and placed individually in plastic breeding cages in a room with a 12 h dark-light cycle and temperature of 24–26 °C. The animals were fed with standard chow (Labofeed, Kcynia, Poland) and had unlimited access to drinking water or paracetamol solution. The paracetamol concentration in the drinking water was adjusted to an average daily intake of 5 mg/kg b.w. (P5) or 15 mg/kg b.w. (P15) for each animal. The dosage was based on earlier observatioons that the average daily water intake by a rat is 20 ± 2 ml. The amount of paracetamol solution consumed and the dose corrections were evaluated daily to reduce the dosage error. The dose 5 and 15 mg/kg of paracetamol were chosen according to clinical practice and previously published studies (*e.g.* Blecharz-Klin et al., 2017). In human neonates doses 10–30 mg/kg b.w. are safe and sufficient to provide appropriate analgesic effect (Irshad et al., 2012; Pacifici and Allegaert, 2014). Use of low doses of paracetamol in the early stages of brain development throughout the whole pregnancy is the safest option that reduce the risk of toxicity and accumulation of the drug. Presented arguments indicate that chosen dosage 5 and 15 mg/kg is perfectly reasonable.

Rodents from the control group received tap water *ad libitum*. Dams were checked daily for offspring, and all offspring were sexed, weighed, and assessed for the presence of congenital defects at postnatal day one (PND1). Pups remained with their mothers for four weeks, and paracetamol administration continued during lactation and after weaning. Male offspring were randomly selected and housed two animals per cage according to group, and drug delivery was continued (P5, $n = 10$; P15, n = 10). Control rat pups continued to receive tap water (Con, n = 10). At Day 60 (PND60), control and paracetamol treated rats underwent social behaviour and episodic memory testing (Fig. 1). Paracetamol administration continued throughout the behavioral tests until the conclusion of the experiment.

All experimental procedures were perfomed in accordance with the recommendations of the Ethical Committee for Animal Experiments at Medical University of Warsaw and in compliance with the ethical standards of Directive 2010/63 EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

2.2. Behavioral assessment

The behavioral response to early paracetamol exposure was estimated by Social Interaction and Novel Object Recognition (NOR) tests, allowing for the evaluation of episodic memory and exploratory behaviour. Animals were habituated to the testing chamber during a 5 min habituation session in the empty plexiglass square box. Behavioral testing began the following day.

2.2.1. Social interaction test

A habituation session was conducted 24 h before testing in the empty test apparatus. Social interaction was assessed in a plexiglass arena (1 × 1 area, 0.3 m height). Rats were placed in the arena opposite a weight-matched partner from a different home cage and observed for 5 min. Social interaction was assessed by the latency of first contact and total time spent interacting, including: sniffing, following, pinning, grooming, or aggressive behaviors. Stress level as assessed by the number of defecations during the session was also evaluated. The arena was cleaned with 10% solution of ethanol after each session.

2.2.2. Novel Object Recognition test (NOR)

Each rat was placed individually in a plexiglass square box (1 × 1 m area, 0.3 m height) with an open top daily for three consecutive days. The task procedure consists of three phases: habituation, familiarization, and choice. In the habituation phase (day 1) each animal was allowed to freely explore the open arena for 3 min in the absence of objects. In the familiarization phase (day 2), two identical objects labeled A1 and A2 (Lego towers) were placed in opposite corners of the box (southwest and northeast) and the time rats spent exploring each object (t_{A1}, t_{A2}) was recorded. Rats were exposed to the arena and allowed to explore for 3 min. All objects were cleaned with 10% solution of ethanol after each session to ensure that behaviour of animals was not guided by odor cues. During the choice phase (day 3) each rat was placed in the southwest corner of the same box containing one familiar object (A1) and a novel object (B) in the northeast corner. Novel and familiar objects had different colors, shapes, and sizes which differentiated them as novel. Object exploration was classified as placement of the rat's head was within 2 cm of any object. The time spent exploring individual objects during the familiarization (t_{A1}, t_{A2}) and choice phases (t_{A1}, t_B), total time spent in exploration of both objects during the familiarization (t_{A1A2}) and choice phases (t_{A1B}), total time of exploration during the second and third day of NOR ($t_{A1A2+A1B}$), discrimination index for familiarization phase - exploration time devoted to the both identical objects [$DI_{A1A2} = (t_{A1} - t_{A2})/(t_{A1} + t_{A2})$], and discrimination index for choice phase - the difference in exploration time for novel *versus* familiar object [$DI_{A1B} = (t_B - t_{A1})/(t_B + t_{A1})$] were all calculated. The global habituation index was also determined by comparing the total time spent in exploring the two objects during the familiarization phase to that spent during the choice phase [$GHI = t_{A1A2}/t_{A1B}$]. The recognition index [$RI = t_B/(t_B + t_{A1A2})$], defined as the time spent investigating the novel object relative to the total object exploration, was also measured.

2.3. Biochemistry

2.3.1. BDNF

Rat pups were decapitated 24 h after behavioral tests. Decapitation is a fast, widely accepted and practiced method of rodents killing. It is a good alternative to lethal anesthesia when the structures of the central nervous system are biochemically analyzed. In this case the use of anesthetic during the procedure of animals killing may affects the neurotransmitters concentration and makes impossible to obtain reliable results.

Brains were removed and the hippocampus, prefrontal cortex and