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Research Report

Maternal caffeine exposure alters neuromotor development and hippocampus acetylcholinesterase activity in rat offspring

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ABSTRACT

The objective of this study was to evaluate the effects of maternal caffeine intake on the neuromotor development of rat offspring and on acetylcholine degradation and acetylcholinesterase (AChE) expression in the hippocampus of 14-day-old infant rats. Rat dams were treated with caffeine (0.3 g/L) throughout gestation and lactation until the pups were 14 days old. The pups were divided into three groups: (1) control, (2) caffeine, and (3) washout caffeine. The washout group received a caffeine solution until the seventh postnatal day (P7). Righting reflex (RR) and negative geotaxis (NG) were assessed to evaluate postural parameters as an index of neuromotor reflexes. An open-field (OF) test was conducted to assess locomotor and exploratory activities as well as anxiety-like behaviors. Caffeine treatment increased both RR and NG latency times. In the OF test, the caffeine group had fewer outer crossings and reduced locomotion compared to control, while the washout group showed increased inner crossings in relation to the other groups and fewer rearings only in comparison to the control group. We found decreased AChE activity in the caffeine group compared to the other groups, with no alteration in AChE

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transcriptional regulation. Chronic maternal exposure to caffeine promotes important alterations in neuromotor development. These results highlight the ability of maternal caffeine intake to interfere with cholinergic neurotransmission during brain development.

1. Introduction

Caffeine (1,3,7-trimethylxanthine) is one of the most widely consumed psychoactive substances worldwide, with approximately 2.5 billion cups (30 mL/cup) of coffee consumed per day (Heckman et al., 2010). This high intake is due to the acute benefits of caffeine in physiological, psychomotor, and cognitive performance (Einöther and Giesbrecht, 2013). However, caffeine is often consumed by childbearing-age women—around 98% of them consume caffeine regularly and 72% continue to consume it during pregnancy (James, 1991). Caffeine easily crosses the placental barrier and thus may affect the development of the fetus (Andersson et al., 2004). Newborns may also be exposed to caffeine in the postnatal period via breastfeeding (Nehlig and Debry, 1994; Santos et al., 2012).

It is a behavioral stimulant found mostly in many beverages and foods, and is not strongly avoided during pregnancy (da Silva et al., 2008; Fredholm et al., 1999). Chronic caffeine intake during pregnancy has been shown to induce a decrease in locomotor activity, learning abilities, and memory in adult rats (Soellner et al., 2009), disturbances in the renin–angiotensin system (Serapião-Moraes et al., 2013), changes in hippocampal acetylcholinesterase (AChE) after 1 g/L of caffeine intake (da Silva et al., 2008), and alterations in fetal brain development (Silva et al., 2013). In both humans and rats, caffeine is rapidly absorbed and crosses the placenta, thereby reaching the fetus. Fetal elimination of caffeine is limited because of a lack of P-450 cytochrome activity, resulting in increased caffeine half-life (Kirkinen et al., 1983; Knutti et al., 1982; Kot and Daniel, 2008). This is further complicated by the fact that caffeine metabolism is slower in pregnant women (Knutti et al., 1982).

Caffeine is a well established adenosine receptor antagonist (Ferre et al., 2008; Sawynok et al., 2010; Sawynok, 2011) that enhances the release of various neurotransmitters, such as dopamine (Fredholm et al., 1999) and acetylcholine (ACh). In addition, caffeine increases the response from dopaminergic receptors due to a negative interaction between adenosine and those receptors (Fredholm and Svenningsson, 2003). Caffeine can also affect ACh levels and its metabolism in the brain (Carter et al., 1995; Murray et al., 1982), and Ach in turn might be involved in the stimulant properties of caffeine (Acquas et al., 2002). In the prefrontal cortex, the cholinergic nerve terminals are involved in attentional processes (Sarter and Bruno, 2002), and ACh turnover is increased after the use of methylxanthines (Murray et al., 1982).

Acetylcholine is a key neurotransmitter involved in cortical activation, attention, memory, learning, pain, control of motor tone and movement, and control of autonomic functions (Herlenius and Lagercrantz, 2004). The cholinergic system is known to be under development during gestation and early postnatal life (Abreu-Villacxa et al., 2011). Studies testing hippocampus-dependent learning and long-term memory showed that caffeine alters the neurogenesis of hippocampal cells in a dose-dependent manner (Han et al., 2007; Wentz and Magavi, 2009). Chronic maternal exposure to high-dose caffeine enhanced AChE activity (42%) without changes in the levels of AChE mRNA transcripts in 21-day-old rats (da Silva et al., 2008). It has also been shown that oral and intravenous administration of caffeine promotes ACh release in the hippocampus and prefrontal cortex (Acquas et al., 2002; Carter et al., 1995). Additionally, long-term consumption of caffeine can disrupt normal hippocampal neurogenesis in adult rats (Han et al., 2007).

Many clinical and experimental studies have been conducted on the behavioral and biological effects of caffeine use on pregnant women (Björklund et al., 2008; Brent et al., 2011; Loomans et al., 2012; Lorenzo et al., 2010; Sengpiel et al., 2013). The prenatal and perinatal periods are crucial and decisive for further ontogenetic development (Horn, 1987); thus, repeated caffeine intake may trigger several neurochemical processes related to anxiety, learning, and lifetime depression during those periods (Li et al., 2012). Considering the susceptibility of the immature brain to adenosine receptor activation and the neuromodulatory role of adenosine on the cholinergic system, our aim was to evaluate the effects of caffeine intake (0.3 g/L) by rat dams during gestation and lactation on the neuromotor development of the offspring and on AChE activity and expression in the hippocampus of 14-day-old rats.

2. Results

The intake of liquids by the dams and the food consumption and weight of the pups were not significantly different between groups (control, caffeine, and washout groups; data not shown).

2.1. *Neuromotor development*

2.1.1. *Righting reflex*

The caffeine and washout-caffeine groups had increased righting reflex latency compared to the control group (ANOVA/SNK, $F_{(2,40)}=6.06$, $p=0.005$; Fig. 1).

2.1.2. *Negative geotaxis*

Prenatal exposure to caffeine significantly affected the negative geotaxis reflex latency time (Fig. 2). Significant differences from the mean were noted over the testing period. Latency was significantly increased in the caffeine and washout-caffeine groups compared to the control group. At the end, the washout group showed a marginally significant improvement, with a