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Increased risk of developing schizophrenia in animals exposed to cigarette smoke during the gestational period

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ABSTRACT

Cigarette smoking during the prenatal period has been investigated as a causative factor of obstetric abnormalities, which lead to cognitive and behavioural changes associated with schizophrenia. The aim of this study was to investigate behaviour and AChE activity in brain structures in adult rats exposed to cigarette smoke during the prenatal period. Pregnant rats were divided into non-PCSE (non-prenatal cigarette smoke exposure) and PCSE (prenatal cigarette smoke exposure) groups. On post-natal day 60, the rats received saline or ketamine for 7 days and were subjected to behavioural tasks. In the locomotor activity task, the non-PCSE + ketamine and PCSE + ketamine groups exhibited increased locomotor activity compared with the saline group. In the social interaction task, the non-PCSE + ketamine and PCSE + ketamine groups exhibited an increased latency compared with the control groups. However, the PCSE + ketamine group exhibited a decreased latency compared with the non-PCSE + ketamine group, which indicates that the cigarette exposure appeared to decrease, the social deficits generated by ketamine. In the inhibitory avoidance task, the non-PCSE + ketamine, PCSE, and PCSE + ketamine groups exhibited impairments in working memory, short-term memory, and long-term memory. In the pre-pulse inhibition (PPI) test, cigarette smoke associated with ketamine resulted in impaired PPI in 3 pre-pulse (PP) intensity groups compared with the control groups. In the biochemical analysis, the AChE activity in brain structures increased in the ketamine groups; however, the PCSE + ketamine group exhibited an exacerbated effect in all brain structures. The present study indicates that exposure to cigarette smoke during the prenatal period may affect behaviour and cerebral cholinergic structures during adulthood.

1. Introduction

A novel hypothesis ranks schizophrenia as a neurodevelopmental disorder that affects 0.5–1.0% of the world's population (Rapoport et al., 2005). Maternal smoking comprises an environmental event investigated as a risk factor for the onset of schizophrenia in adulthood, which affects cognitive and behavioural disorders (Baguelin-Pinaud et al., 2010). Preclinical studies have demonstrated that exposure to nicotine induces changes in the developmental neurochemical markers of dopamine (DA) and may cause hyperactivity in offspring (Azam et al., 2007). In addition, a study published in our laboratory demonstrated that exposure to cigarette smoke during gestation causes persistent changes in lipids and proteins, as well as deoxyribonucleic acid (DNA) damage, which leads to oxidative stress in cells (Fraga et al., 2011).

Acetylcholine (ACh) is an excitatory neurotransmitter synthesized in presynaptic neurons by choline acetyltransferase (ChAT); it acts on the neuromuscular junction and binds to muscarinic or nicotinic receptors. It has a main role in motor, cognitive and memory functions (Voss et al., 2008). Acetylcholinesterase (AChE) is a regulatory enzyme that controls the transmission of a nerve pulse through a cholinergic synapse

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; ANOVA, analysis of variance; BChE, butyrylcholinesterase; ChAT, choline acetyltranferase; CNS, central nervous system; DA, dopamine; DNA, deoxyribonucleic acid; DTNB, 5,5-dithiobis-2-nitrobenzoic acid; EDTA, ethylenediamine tetraacetic acid; MK-801, dizocilpine maleate; nAChR, nicotinic acetylcholine receptor; nAChRs, α7-α7 nicotinic acetylcholine receptors; NIH, National Institutes of Health; NMDAR1, NMDA receptor subunit 1; P, pulse; PCSE, prenatal cigarette smoke exposure; PND, postnatal day; PP, pre-pulse; PPI, pre-pulse inhibition; SBNeC, Brazilian Society for Neuroscience and Behavior; UNESC, Universidade do Extremo Sul Catarinense.

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via hydrolysis and ACh inactivation (Soreq and Seidman, 2001). In schizophrenic patients, cognitive dysfunctions are often present and have been related to changes in the cholinergic system (Voss et al., 2008).

Evidence suggests there is a relationship between the cholinergic and glutamatergic systems. A study conducted by Colgin et al. (2003) indicated that cholinergic signals induce excitatory activity in glutamate, which facilitates memory acquisition. Furthermore, the involvement of the cholinergic system in the physiopathology of mental disorders has been well established. Changes in ACh receptors occur in schizophrenia. The habit of smoking is significantly increased in schizophrenics compared with the general population, and this difference appears to be significant even in patients with another psychiatric disorder (de la Rubia et al., 2003; Aguilar et al., 2005; McCreadie, 2002). These patients also manifest more severe positive symptoms, which indicate the involvement of nicotinic receptors in this disorder (Aguilar et al., 2005; Kalman et al., 2005; Ziedonis et al., 1994). In this context, studies indicate that individuals chronically exposed to cigarette smoke in the prenatal period had an increased risk of developing cognitive deficits, hyperactivity and conduct disorder during adolescence (Abreu-Villaca et al., 2010; Liu and Zhao, 2004).

Neurodevelopmental issues may affect children whose mothers are subjected to stress during pregnancy (Brixey et al., 1993; O'Donnell, 2011). Smoking during the gestational period may trigger obstetric abnormalities and low weight shortly after birth, which lead to cognitive and behavioural changes during childhood (Baguelin-Pinaud et al., 2010). This change in early neurodevelopment may modify the organization of axonal connections in the synaptic projections that affect neuronal cell proliferation, migration and apoptosis (Van de Berg et al., 2002), which are necessary for the development of the central nervous system (CNS) (Stathopoulou et al., 2013).

Based on evidence that exposure to cigarette smoke during the prenatal period is directly related to changes in neurodevelopment and consequently related to the pathophysiology of schizophrenia, data in the literature indicate that an inappropriate intrauterine environment favours the incidence of psychiatric disorders in offspring; moreover, the synergism between glutamatergic and cholinergic systems may comprise a mechanism that underlies the symptoms of this disorder.

Overall, these findings have not been completely explained, and additional studies are warranted. Thus, in this study, we hypothesized that adult rats exposed to cigarette smoke during the prenatal period will exhibit an increased susceptibility to trigger schizophrenia-like behaviour and changes in the cholinergic system during adulthood.

2. Materials and methods

2.1. Animals

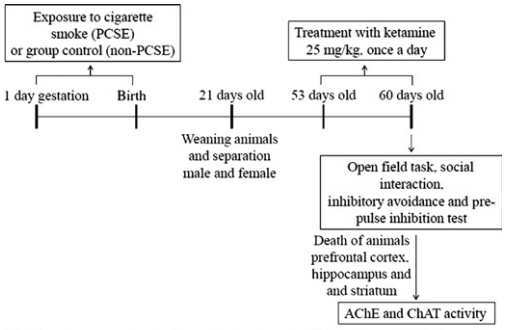
All experimental procedures that involved animals were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the recommendations of the Brazilian Society for Neuroscience and Behavior (SBNeC). These procedures were approved by the local Ethics Committee of the Universidade do Extremo Sul Catarinense (UNESC) under protocol number 118/2012. Pregnant female Wistar rats (3–4 months old, 250–280 g weight) were obtained from the local breeding colony and were individually housed with sawdust bedding and ad libitum access to food and water.

2.2. Prenatal cigarette smoke exposure (PCSE)

Dams (pregnant females, n = 25) were randomly assigned to one of two treatment groups (n = 15 per group: 1) non-PCSE (not exposed to cigarette smoke) or 2) PCSE (exposed to cigarette smoke throughout the pregnancy). Each rat was exposed to the smoke of 12 commercially filtered cigarettes per day (a total of 8.0 mg of tar and 0.6 mg of nicotine) for a period of 22 consecutive days. The animals were placed in an inhalation chamber (dimensions of 40 × 30 × 25 cm) inside a fume extraction cabinet. Cigarettes were coupled to a 60 mL plastic syringe; thus, puffs could be drawn in, expelled, and aspirated with this syringe (20 puffs of 50 mL). Each puff was immediately injected into the chamber. The animals were maintained in this smoke-air condition (3%) for 6 min. The cover was subsequently removed from the inhalation chamber, and the smoke was evacuated within 1 min by activating the cabinet exhaust fan. The dams were immediately exposed to cigarette smoke from a second cigarette for 6 min. This process was repeated three times per treatment, and these four cigarette treatments were performed three times per day (morning, noon, and afternoon), which resulted in 72 min of cigarette smoke that produced 300 mg/mm^3 of total particulate matter in the chamber.

2.3. Ketamine treatment

The rats were weaned on postnatal day (PND) 21. On PND 60, the rats were randomly assigned to one of the following treatment groups: 1) non-PCSE + saline; 2) non-PCSE + 25 mg/kg ketamine; 3) PCSE + saline; and 4) PCSE + 25 mg/kg ketamine. All animals received a single intraperitoneal injection, once a day, for 7 days, according to the treatment protocols, at a volume of 1 mL/100 mg of body weight per injection (de Oliveira et al., 2011). After the last injection of ketamine, the rats were subjected to behavioural tasks according to protocols described by de Oliveira and Hunt (de Oliveira et al., 2011; Hunt et al., 2006).



Timeline of exposure to cigarette smoke, treatment with ketamine and behaviour tasks

2.4. Open field task

The animals were assessed in the open field test 30 min after the final ketamine injection. The test was performed in an arena with dimensions of 50 × 25 × 50 cm. The locomotor activity was measured for 15 min using a computerized system (Activity Monitor, Insight Laboratory Equipment, Ribeirão Preto, SP). The locomotor activity was quantified as the distance covered by the animal (in cm) in blocks of 5 min. The distance covered was calculated as the sum of the position changes monitored by the system.

2.5. Social interaction

Impaired social interaction is a characteristic behaviour of animal models of autism spectrum disorders and schizophrenia (Dicicco-Bloom et al., 2006; Mohn et al., 1999; Schneider and Przewlocki, 2005). The animals (n = 12) were tested in ambient light/dark and unfamiliar conditions in an open field apparatus. On the day of the experiment, the animals were socially isolated in a plastic box that measured 43 × 28 × 15 cm for 3 h prior to the experiment. The task consisted of placing two animals in the same group randomized into cages for 15 min. The social behaviour was assessed for a pair of animals; thus, the behaviours of individual animals were not analysed (Schneider and Przewlocki, 2005). The latency to the first interaction,