Behavioural Brain Research 307 (2016) 137–144

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Short-term exposure and long-term consequences of neonatal exposure to Δ^9-tetrahydrocannabinol (THC) and ibuprofen in mice

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HIGHLIGHTS

- Examined drug effects of short-term THC and ibuprofen exposure during a critical period of brain development in mice.
- Neonatal single-dose exposure to THC induced altered adult spontaneous behavior and reduced habituation capability in a dose-response related manner.
- Ibuprofen exposure during brain growth spurt, using the same behavioral model, did not affect adult behavior or habituation capability.

ARTICLE INFO

Article history:
Received 16 February 2016
Received in revised form 30 March 2016
Accepted 2 April 2016
Available online 4 April 2016

Keywords:
Δ^9-Tetrahydrocannabinol
Ibuprofen
Analgesics
Developmental neurotoxicity
Cognitive function

ABSTRACT

Both Δ^9-tetrahydrocannabinol (THC) and ibuprofen have analgesic properties by interacting with the cannabinoid receptor type 1 (CB1R) and the cyclooxygenase (COX) systems, respectively. Evaluation of these analgesics is important not only clinically, since they are commonly used during pregnancy and lactation, but also to compare them with acetaminophen, with a known interaction with both CB1R and the COX systems. Short-term exposure of neonatal rodents to acetaminophen during the first weeks of postnatal life, which is comparable with a period from the third trimester of pregnancy to the first years of postnatal life in humans, induces long-term behavioral disturbances. This period, called the brain growth spurt (BGS) and is characterized by series of rapid and fundamental changes and increased vulnerability, peaks around postnatal day (PND) 10 in mice. We therefore exposed male NMRI mice to either THC or ibuprofen on PND 10. At 2 months of age, the mice were subjected to a spontaneous behavior test, consisting of a 60 min recording of the variables locomotion, rearing and total activity. Mice exposed to THC, but not ibuprofen, exhibited altered adult spontaneous behavior and habituation capability in a dose-dependent manner. This highlights the potency of THC as a developmental neurotoxicant, since a single neonatal dose of THC was enough to affect adult cognitive function. The lack of effect from ibuprofen also indicates that the previously seen developmental neurotoxicity of acetaminophen is non-COX-mediated. These results might be of importance in future research as well as in the ongoing risk/benefit assessment of THC.

1. Introduction

Cannabis is one of the most commonly used psychoactive drugs with over 180 million users worldwide in the ages 15–64 years [1]. In some countries cannabis use has a prevalence of up to 40% in adults [2]. Cannabis is also one of the most frequently used drugs during pregnancy and lactation [1,2]. In recent years, there has been a re-emergence in public media's attention to cannabis, partly due to the medical benefits of the drug. This increased attention is being further fueled by a growing opinion that cannabis is considered relatively harmless. Due to the hydrophobic nature of Δ^9-tetrahydrocannabinol (THC), the active ingredient in cannabis, it can reach the fetal brain across the placenta [3] and blood brain barrier [4] and may therefore affect fetal brain development. THC is also present in milk of breast feeding mothers after cannabis consumption [5]. THC binds to the cannabinoid receptor type 1 (CB1R), one of two major receptors of the endocannabinoid system (ECS) [6–8]. Exposure to THC during human brain development, most

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http://dx.doi.org/10.1016/j.bbr.2016.04.001
0166-4328/

often because of maternal smoking of marijuana, has been shown to be associated with impairment of higher cognitive function later in life, manifested as working memory deficits, learning disabilities and impaired attention [9–11]. Similar effects, such as increased locomotion and impaired learning and memory, have also been shown in animal models after prenatal exposure to THC or the synthetic CB1R receptor agonist WIN 55,212-2 (WIN) [12–15].

The neurotoxic effects caused by affecting the ECS during brain development is also highly interesting since acetaminophen, one of the most commonly used analgesics and antipyretics, has been suggested to interact with the CB1R through its metabolite AM404 [16,17]. THC, like AM404, exhibits analgesic properties, which in part, can be attributed to the common interaction with the CB1R. Acetaminophen exposure during brain development has been shown to affect behavior later in life in both preclinical [18] and human studies [19–21]. In the preclinical study on mice, a single day acetaminophen administration during neonatal brain development was shown to affect spontaneous behavior in adulthood [18]. Here, a hyperactive adult phenotype was observed, which is in agreement with the neurodevelopmental outcome after THC exposure, therefore further suggesting a possible CB1R-mediated acetaminophen-induced neurotoxic effect. Whether the effects of acetaminophen exposure during brain development is due to its effects on the ECS or through its effects on other systems, e.g. the cyclooxygenase (COX) system, is however yet unknown.

The COX system can be affected by other analgesics and antipyretics, such as ibuprofen, which belongs to the group of non-steroidal anti-inflammatory drugs (NSAID), and like acetaminophen, is one of the most used over-the-counter (OTCs) drugs treating for pain and fever during pregnancy and early life [22]. The mechanism of action of ibuprofen largely depends on its inhibition of COX [23] and the main products of COX activity are the prostaglandins, which in turn are lipid mediators important in modulation of many brain activities. For instance, the activity of COX-2, one of two isoforms of COX, is involved in synaptic activity and plasticity, hippocampal long-term potentiation (LTP) and maturation of the brain (reviewed: [24,25]). In one of the aforementioned epidemiology studies of acetaminophen, ibuprofen was ruled out as a possible confounding cause of the developmental neurotoxic effects [19]. Still it is important to evaluate the potential *in vivo* developmental neurotoxicity of ibuprofen and compare it to other types of analgesic and antipyretic agents. By using THC, with a known interaction with the ECS and ibuprofen, with a known interaction with the COX system, this study might therefore also give clues about the neurotoxic mechanism of acetaminophen.

The developing human brain is more susceptible to injury caused by xenobiotics than the adult brain [26,27]. Due to the complexity of mammalian brain development, injuries during this period are more likely to be permanent [27,28]. During brain development there is a specifically vulnerable period called the brain growth spurt (BGS), characterized by a series of rapid and fundamental changes, including maturation of dendritic and axonal outgrowth, synaptogenesis, establishment of neuronal connections, proliferation of glia cells and myelination [27,29]. In humans, the BGS begins in the third trimester of pregnancy and continues throughout the first 2 years of life, with a peak around birth. In mice and rats however, this period is completely postnatal, spanning the first 3–4 weeks of neonatal life and peaking around postnatal day (PND) 10 [27,29,30] thereby providing an excellent opportunity to pinpoint this interesting period and simulate indirect late prenatal exposure and both indirect and direct postnatal exposure.

Interestingly, the ECS is already present during early brain development and is important in progenitor cell proliferation and differentiation, synaptogenesis, neuronal migration, correct axonal and neurite outgrowth [31–35]. There is already a large body of evidence of developmental neurotoxic effects of chronic exposure to THC, with long-lasting effects on locomotor activity, cognitive function, emotional disturbances and increased sensitivity to other drugs (summarized in review [36]). However, preclinical studies on the long-term effects of a neonatal single-dose exposure of THC in rodents, has to the best of our knowledge, not been evaluated before. Moreover, there is lack of preclinical data of the long-term effects, when affecting the COX system, which is surprising, since one of the most used painkiller during pregnancy and early life, ibuprofen, is affecting this system.

In our behavioral model we assess spontaneous behavior, which explores the subject's capability of habituation, in their own home environment (described in more detail in Section 2.3). Spontaneous behavior recordings is a highly sensitive method, which has previously revealed persistent adult neurobehavioral deficits, manifested as disruption of habituation and altered spontaneous behavior, in rodents after short-term exposure during the BGS (particularly on PND 10) of both commonly used anesthetics and analgesics, such as acetaminophen, ketamine and propofol [18,37,38], as well as alcohol [39] and environmental agents such as DDT, PCBs, PBDEs and PFCs [40–43]. Exposures occurring before or after the peak of the BGS do usually not induce behavioral or cognitive effects and in the rare occasion where effects are seen, they are not persistent, further emphasizing the critical window during the peak of the BGS [44,45].

In light of the ongoing debate regarding legalization of cannabis, the fact that it's the most frequently used drug during pregnancy and lactation together with the normalization of its usage, continuous evaluation of the adverse effects associated with exposure to THC is of high importance. The evaluation of ibuprofen is also interesting and important for two reasons. Firstly, it's one of the commonest drugs used by pregnant women where little is known about the long-term effects if exposed during brain development, and secondly, for comparative mechanistic purposes with acetaminophen (which has an interaction with both COX and CB1R). The aim of this study was therefore to investigate if short-term exposure to THC and ibuprofen, during the peak of the BGS, could affect spontaneous behavior and cognitive function in adult mice in a dose-response related manner.

2. Material and methods

Experiments were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), after approval from the local ethical committee (Uppsala University and Agricultural Research Council) and by the Swedish Committee for Ethical Experiments on Laboratory Animals.

2.1. Animals

Pregnant NMRI mice were purchased from B&K, Sollentuna, Sweden, and were housed individually in plastic cages in temperature-controlled environment (22 °C) and maintained in light-controlled room (12 h light/dark cycle). All experimental animals had free access to standardized pellet food (Lactamin, Stockholm, Sweden) and tap water. The pregnant NMRI mice were checked for birth once daily (06.00 PM). Litter sizes were adjusted, within 48 h after birth, to 10–12 pups of both sexes by euthanizing excess pups. At the age of ~4 weeks male offspring were separated from their female siblings, who were euthanized, and were kept with their male siblings from each treatment group. Litters contained 4–7 animals. Only male mice were used in this study.

2.2. Chemicals and exposure

THC (Dronabinol, LGC, DAC Quality) was generously provided by Pronexa and was dissolved in peanut oil/egg lecithin (Merck,