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Prenatal exposure to the cannabinoid receptor agonist WIN 55,212-2 increases glutamate uptake through overexpression of GLT1 and EAAC1 glutamate transporter subtypes in rat frontal cerebral cortex

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Abstract

Prenatal exposure to the CB1 receptor agonist (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone) mesylate (WIN) at a daily dose of 0.5 mg/kg, and Δ^9-tetrahydrocannabinol (Δ^9-THC) at a daily dose of 5 mg/kg, reduced dialysate glutamate levels in frontal cerebral cortex of adolescent offspring (40-day-old) with respect to those born from vehicle-treated mothers. WIN treatment induced a statistically significant enhancement of V_{max} L-[^{3}H]glutamate uptake, whereas it did not modify glutamate K_m, in frontal cerebral cortex synaptosomes of adolescent rats. Western blotting analysis, performed either in membrane proteins derived from homogenates and in proteins extracted from synaptosomes of frontal cerebral cortex, revealed that prenatal WIN exposure enhanced the expression of glutamate transporter 1 (GLT1) and excitatory amino acid carrier 1 (EAAC1). Moreover, immunocytochemical analyses of frontal cortex area revealed a more intense GLT1 and EAAC1 immunoreactivity (ir) distribution in the WIN-treated group. Collectively these results show that prenatal exposure to the cannabinoid CB1 receptor agonist WIN increases expression and functional activity of GLT1 and EAAC1 glutamate transporters (GluTs) associated to a decrease of cortical glutamate outflow, in adolescent rats. These findings may contribute to explain the mechanism underlying the cognitive impairment observed in the offspring of mothers who used marijuana during pregnancy.

Keywords: Glutamate outflow; Microdialysis; Glutamate uptake; GLT1 glutamate transporter; EAAC1 glutamate transporter; Frontal cerebral cortex; Maternal marijuana consumption

1. Introduction

Among the social problems connected with the use of marijuana, the large scale consumption of marijuana in pregnant women (Fried and Smith, 2001; Fried et al., 2003) has increased urgency to understanding its effects on the foetus. However, despite the existence of numerous studies, the results obtained are often controversial and conclusions remain still uncertain. Although marijuana is not a classic human teratogen compound, recent findings suggest that marijuana exposure may have subtle negative effects on neurobehavioural

Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; EAAC1, excitatory amino acid carrier 1; EAAT4, excitatory amino acid transporter 4; EAAT5, excitatory amino acid transporter 5; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GD, day of gestation; GFAP, glial fibrillary acidic protein; GLAST, glutamate/aspartate transporter; GLT1, glutamate transporter 1; GluTs, glutamate transporters; HPRT, hypoxanthine-phosphoribosyl-transferase; NGS, non immune goat serum; P2, synaptosomal pellet; PBS, phosphate-buffered saline; PFA, paraformaldehyde; SYT1, synaptotagmin I; WIN, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinyl-methyl)-pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone)mesylate.

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outcomes of the offspring, including sleep disturbances, impaired visual problem-solving, hyperactivity and inattention (Fried and Smith, 2001; Kozer and Koren, 2001). That cannabis might exert some deleterious effect on the foetus could be realistic since the active ingredients of marijuana extract can be transferred from the mother to the foetus through the placental blood during gestation (Hutchings et al., 1989). In line with this view, previous studies reported that developmental exposure to Δ^9-THC, the main psychoactive component of marijuana extract, may cause in the offspring transient and permanent changes in the function of several neuronal systems like dopaminergic (Walters and Carr, 1988; Rodriguez de Fonseca et al., 1991; Bonnin et al., 1996), serotonergic (Molina-Holgado et al., 1996), GABAergic (Garcia-Gil et al., 1999) and opioidergic ones (Vela et al., 1998). Furthermore, more recently it has been demonstrated that prenatal cannabinoid exposure is also associated with changes in glutamate transmission in different brain regions of the offspring (Mereu et al., 2003; Antonelli et al., 2004, 2005). In fact, prenatal exposure to the CB1 receptor agonist WIN induces cognitive impairment and emotional reactivity changes associated with a reduced basal and K^+-evoked in vivo glutamate outflow in frontal cerebral cortex of 90-day-old offspring (Antonelli et al., 2004). High-affinity uptake is the major mechanism by which the CNS regulates the extracellular levels of glutamate, the main excitatory neurotransmitter in the cerebral cortex (Danbolt, 2001). To date, five different isoforms of GluTs have been identified: glutamate/aspartate transporter (GLAST), glutamate transporter 1 (GLT1), excitatory amino acid carrier 1 (EAAC1), excitatory amino acid transporter 4 (EAAT4) and excitatory amino acid transporter 5 (EAAT5) (Danbolt, 2001). GLT1 is the most abundant glutamate transporter and represents about 1% of the total brain membrane proteins, with highest concentrations in the hippocampus and cerebral cortex (Rothstein et al., 1994; Samuelsson et al., 2000). In addition, the activity of GLT1 is responsible for the greatest proportion of total glutamate transport and its functional inactivation raises extracellular glutamate levels (Rothstein et al., 1996; Tanaka et al., 1997). The highest concentration of GLAST is found in the Bergmann glia of the cerebellar molecular layer (Lehre et al., 1995), but it is also expressed in the cerebral cortex (Rothstein et al., 1994). In the neocortex, EAAT4 and EAAT5 expression is poor or absent (Danbolt, 2001) and therefore their contribution to total cortical glutamate uptake is extremely small, whereas EAAC1 is expressed (Lehre et al., 1995) and mediates a sizeable fraction of total glutamate uptake (Rothstein et al., 1996). Based on the above considerations, the main aim of the present study was to assess whether prenatal exposure to the CB1 receptor agonist WIN could affect glutamate uptake and consequently to induce glutamate outflow changes. Therefore we investigated whether prenatal exposure to WIN may affect GLT1, EAAC1 and GLAST expression and functional activity. All experiments have been performed in frontal cerebral cortex, one of the major site subserving cognition and memory processing (Courtney et al., 1998; Fuster, 2000) of adolescent rats (40-day-old) since previous human studies provided evidence that adolescents born to women who used marijuana during pregnancy exhibit deficits in cognitive functions (Fried and Watkinson, 2001; Fried and Smith, 2001; Fried et al., 2003; Smith et al., 2004).

2. Materials and methods

2.1. Animal care

Experiments were carried out in strict accordance with the guidelines released by the Italian Ministry of Health (D.L. 116/92) and (D.L. 111/94-B), the Declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the National Institutes of Health (USA).

2.2. Animals and exposure conditions

Primiparous Wistar female rats (Harlan SRC, Milan, Italy) weighing 250–280 g were initially housed for 1 week at constant room temperature (20 ± 1 °C), humidity (60%) and exposed to a light cycle of 12 h/day (08:00 to 20:00), with food and water available ad libitum. Pairs of females were then placed with single male rats in the late afternoon. Vaginal smears were taken the following morning at 09:00 h. The day on which sperm were present was designated as the day of gestation (GD) 0. Either WIN or Δ^9-THC were administered daily from GD 5 through GD 20. WIN was administered at 0.5 mg/kg, a dose not affecting dam weight gain, pregnancy length and litter size at birth (Mereu et al., 2003). The drug was suspended in 0.3% Tween 80–saline, as previously described (Tanda et al., 1997) and injected subcutaneously at the volume of 1 ml/kg. Δ^9-THC, at the dose of 5 mg/kg in sesame oil, was administered through a buccopharyngeal cannula. This dose is an extrapolation from current estimates of moderate exposure to Δ^9-THC in humans, correcting for differences in route of administration and body surface area. In both cases, control rats were treated with the respective vehicle. Litters were reduced to a standard size of six male pups per litter (when possible) within 24 h after birth. Litters from WIN or Δ^9-THC-vehicle group (control) or WIN or Δ^9-THC-exposed (WIN, THC, respectively) group, were then assigned (six pups per litter) to non-exposed mothers whose pups were born on the same day. Data were collected only from male pups whose mothers were exposed either to control or to WIN or THC during pregnancy. Pups were weaned at 21 days of age. Thereafter, rats were housed in groups of five animals per cage.

2.3. Microdialysis experiments

2.3.1. Surgery

In vivo experiments were performed in the offspring of WIN, THC, and control dams, at the age of 40 days. Briefly, the animals, kept under halothane anaesthesia (1.5% mixture of halothane/air), were mounted in a David Kopf stereotaxic frame and a microdialysis was implanted into the right frontal cerebral cortex (2 mm dialysing membrane length) using the following stereotaxic coordinates (A: +3.5, L: ±2.8; V: −3.5) (Paxinos and Watson, 1986). After the implantation, the probe was secured to the skull with methacrylic cement and 36 h later microdialysis was performed.

2.3.2. Experimental protocol

On the day of the microdialysis experiment, the probe was perfused with a modified cerebrospinal fluid: NaCl 148 mM; KCl 2.7 mM; $CaCl_2$ 1.2 mM; $MgCl_2$ 0.85 mM; glucose 2.7 mM, at a constant flow rate of 2 μl/min by using a microinfusion pump. Once basal glutamate levels were constant (300 min after the onset of perfusion), four consecutive perfusate fractions were collected every 20 min.

2.4. Histology

At the end of each experiment, the location of the probe was verified in 30-μm-thick coronal cryostat sections. Only those animals in which the probe was