Neuroscience 172 (2011) 139–152

MATERNAL EXPOSURE TO THE CB1 CANNABINOID AGONIST WIN 55212–2 PRODUCES ROBUST CHANGES IN MOTOR FUNCTION AND INTRINSIC ELECTROPHYSIOLOGICAL PROPERTIES OF CEREBELLAR PURKINJE NEURONS IN RAT OFFSPRING

M. SHABANI,[a,b] N. HOSSEINMARDI,[a] M. HAGHANI,[a] V. SHAIBANI[b] AND M. JANAHMADI[a]*

[a]*Neuroscience Research Centre and Department of Physiology, Medical School, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Islamic Republic of Iran*

[b]*Neuroscience Research Centre and Physiology Research Centre, Kerman University of Medical Sciences, Kerman, Islamic Republic of Iran*

Abstract—The cerebellum, which controls coordinated and rapid movements, is a potential target for the deleterious effects of drugs of abuse including cannabis (i.e. marijuana, cannabinoids). Prenatal exposure to cannabinoids has been documented to cause abnormalities in motor and cognitive development, but the exact mechanism of this effect at the cellular level has not been fully elucidated. Previous studies indicate that cannabinoids are capable of modulating synaptic neurotransmission. In addition to altering synaptic activity, cannabinoid exposure may also change intrinsic neuronal properties. In the present study several different approaches including behavioral assays, extracellular field potential recordings and whole-cell patch clamp recordings, were used to address whether maternal exposure to the CB1 cannabinoid receptor agonist WIN 55–212–2 (WIN) affects the intrinsic electrophysiological properties of Purkinje neurons. WIN treatment of pregnant rats produced a significant decrease in the rearing frequency, total distance moved and mobility of the offspring, but significantly increased the time of the righting reflex, the grooming frequency and immobility. Neuromotor function, as assessed in the grip test and balance beam test, was also significantly impaired in prenatally WIN-treated group. Prenatal exposure to WIN increased the amplitude of population spikes (PS) recorded from the cerebellar Purkinje cell layer of offspring following synaptic blockage. WIN treatment of pregnant rats also profoundly affected the intrinsic properties of Purkinje neurons in offspring. This treatment increased the firing regularity, firing frequency, amplitude of afterhyperpolarization (AHP), the peak amplitude of action potential and the first spike latency, but decreased significantly the time to peak and duration of action potentials, the instantaneous firing frequency, the rate of rebound action potential and the voltage "sag" ratio. These results raise the possibility that maternal exposure to cannabinoids may profoundly affect the intrinsic membrane properties of cerebellar Purkinje neurons of offspring by altering the membrane excitability through modulation of intrinsic ion channels.

Key words: cannabinoids, cerebellum, intrinsic excitability, prenatal exposure, Purkinje neurone, whole cell patch.

Cannabinoids, which are commonly used in the form of marijuana and hashish, influence prenatal and postnatal biological processes including the function of the nervous system; however, the diverse cellular effects of their prenatal exposure on offspring are still not well defined. Cannabinoid type 1 (CB1) receptor, which is widely distributed in many brain regions including the cerebellum, has been shown to be involved in a variety of important functions during both prenatal and postnatal life. Several lines of evidence from animal and human studies indicate that prolonged prenatal exposure to cannabinoid CB1 receptor agonists induces numerous and complex effects on cognitive and motor functions (Ranganathan and D'Souza, 2006; Pattij et al., 2008; Huizink and Mulder, 2006; Garcia-Gil et al., 1999). Therefore, the cerebellum, which is involved in motor coordination and learning new motor skills, could be one of the brain regions most vulnerable to prenatal exposure to cannabinoids. Purkinje neurons, which constitute the sole output of all motor coordination in the cerebellar cortex, have been shown to trigger release of endogenous cannabinoids in response to elevated Ca^{2+} and thereby inhibit presynaptic calcium influx and suppress transmitter release (Kreitzer and Regehr, 2001). Depolarization-induced retrograde inhibition of both excitatory and inhibitory synapses in the cerebellar cortex mediated by cannabinoid receptors has been well documented (Kreitzer and Regehr, 2001; Szabo et al., 2006). More recently, Goonawardena et al. (in press) using the extracellular single unit recording method have reported that postnatal administration of cannabinoids alters firing behavior of hippocampal neurons. They showed that cannabinoid receptors reduce neuronal firing rates and change burst characteristics. To the best of our knowledge, however, there have been no reports about the prenatal effect of cannabinoids on intrinsic neuronal excitability. In the present study, we have addressed the question whether the intrinsic electrophysiological properties of cerebellar Purkinje neurons of offspring may be affected by maternal exposure to the cannabinoid agonist WIN 55,212–2 using the patch clamp technique in a whole-cell configuration. As previously documented, any alterations

*Corresponding author. Tel: +0098-21-44218549; fax: +0098-21-22439971.
E-mail address: mjanahmadi@yahoo.com or Janahmadi@sbmu.ac.ir (M. Janahmadi).

Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; CB1, cannabinoid type 1 receptor; WIN, WIN 55–212–2; PN, postnatal day; PS, population spikes; T, threshold intensity; PCs, Purkinje cells.

0306-4522/11 $ - see front matter © 2011 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2010.10.031

in the intrinsic excitability and firing behavior of Purkinje cells (PCs) may be associated with profound changes in motor activity and movement coordination (Walter et al., 2006; Shakkottai and Paulson, 2009; Janahmadi et al., 2009; Perkins et al., 2010). Neuronal firing properties are determined by synaptic inputs and intrinsic membrane functions such as biophysical characteristics of intrinsic ionic conductances (Kaczmareck and Levitan, 1987). Alterations in the firing frequency and pattern may be achieved either by altering the amount of presynaptic neurotransmitter release or by modifying postsynaptic ionic conductances. Such changes, in turn, are expected to produce alterations in neuronal responsiveness including neural coding and sensory processing or generating motor inputs (Kaczmareck and Levitan, 1987), which would be likely associated with changes in animal behavior.

The cellular mechanisms of prenatal cannabinoid exposure on neuronal excitability of offspring have not been fully elucidated, although several behavioral effects have been described (Moreno et al., 2005; Antonelli et al., 2005; Ferraro et al., 2009). Maternal exposure to the cannabinoid CB1 receptor agonist WIN 55,212–2 causes learning and memory disruption associated with a reduction in cortical glutamatergic neurotransmission and NMDA receptor activity in rat offspring (Mereu et al., 2003; Antonelli et al., 2005). There is also clinical evidence showing abnormal motor activity in children of mothers who used marijuana during pregnancy (Fried et al., 1992; Goldschmidt et al., 2000). In addition, experimental animal data have shown that offspring of dams exposed to WIN exhibit abnormal motor performance during infantile and juvenile but not adult periods (Mereu et al., 2003).

Cannabinoid consumption during pregnancy has also been shown to interfere with proper maturation of the brain (Fergusson et al., 2002).

Based on the above findings, the main goal of the present study was to test the hypothesis that maternal exposure to the selective CB1 cannabinoid receptor agonist WIN leads to alterations in the intrinsic electrophysiological properties of rat cerebellar Purkinje neurons using behavioral and electrophysiological approaches.

EXPERIMENTAL PROCEDURES

Animals

All procedures for the care and the use of experimental animals were approved by the Institutional Ethics Committee (IEC) at the University of Shahid Beheshti Medical Sciences. Animal discomfort was minimized, and the number of animals used was the minimum necessary for meaningful interpretation of data. Primiparous Wistar female rats (Pasteur Institute, Karaj, Islamic Republic of Iran) weighing 200–250 g were housed at 22 °C and maintained on a 12:12-h light/dark cycle with free access to food and water. After 1-week of acclimatization, female rats were mated to males of the same strain in the late afternoon. Vaginal smears were taken the following morning at 9:00 AM. Day 1 of pregnancy was designated the day sperm was present in the vaginal smear. Pregnant rats were then randomly assigned to four groups (n=6 per group): control, sham-treated and two groups receiving WIN (0.5 or 1 mg/kg, suspended in 1% Tween 80/saline solution and injected s.c. daily at a volume of 1 ml/kg; six females per group) from the fifth day until the end of pregnancy. The sham treated rats received 1% Tween 80/saline solution injected s.c. daily at a volume of 1 ml/kg. Pups were weaned at 21 days of age. Body weights of dams were determined weekly during pregnancy. The litter size at birth and the length of pregnancy were also measured. Measurements were taken during daylight (between 8:00 and 16:00 h).

Assessment of motor activity

Open field test. At 22, 36 and 50 days of age the horizontal and vertical activities of the male rat pups from each group were recorded for a period of 5 min and analysed using Ethovision software (version 3.1), a video tracking system for automation of behavioral experiments (Noldus Information Technology, the Netherlands). The apparatus consisted of a square arena ($42\times42\times30$ cm^3) made of black wood. The arena was divided into nine equally sized squares that allowed the definition of central (one squares) and peripheral (eight squares) parts. At the beginning of the session, each pup was placed in the centre of the arena and its activity was recorded for 5 min and the following behavioral parameters were then scored: total distance moved (TDM, cm); total duration mobility(s) and immobility (s); and frequency of rearing (as a measure of vertical activity) and grooming (rubbing the body with paws or mouth and rubbing the head with paws). At the end of each session, rat offspring were removed from the open field and the experimental chamber was thoroughly cleaned with a damp cloth and dried.

Rotarod/accelerated test. On postnatal days of 22 and 36, the accelerating rotarod (Hugo Sachs Electronik, Germany) test was performed to assess motor coordination and balance of rat pups. With a minimum speed of 5 rpm, each pup was placed on a rotating rod (8.9 cm long and 3.8 cm in diameter) covered with rubber and left for 5 min for habituation. After three training runs, the pup was tested. The speed of the rotated rod was slowly increased from 5 to 20 rpm over the course of 5 min. Pups were given three trials on each experimental day of a maximum time of 300 s (5 min) with a 30 min inter-trial rest interval, and the length of time each animal was able to maintain its balance walking on top of the rod was measured.

Righting reflex. To assess gross motor function and coordination of the pups, a righting reflex was measured from the second day of age. Each rat pup was turned onto its back and the latency to stably place all four paws on the surface was recorded.

Wire grip test. This test was performed according to van Wijk et al. (2008) and used to assess the muscle strength and balance. Rat was suspended with both forepaws on a horizontal steel wire (60 cm long, diameter 7 mm). The animal was held in a vertical position when its front paws were placed in contact with the wire. When the rat grasped the wire, it was released, and the latency to fall was recorded with a stopwatch. Rats were randomly tested and each animal was given one trial.

Balance beam test. The balance beam test was used to measure the muscle coordination of animal. A horizontal narrow, smooth-surface, round wooden beam (diameter 2.6 cm and length 60 cm) was suspended 50 cm above a foam-padded cushion. One side of the beam, a light bulb was placed, in order to trigger the animals to move. Animals were placed with all four paws in the middle of the beam with its head toward the illuminated side (van Wijk et al., 2008). Each rat was given one trial and the latency to fall was recorded.

Electrophysiological studies

Extracellular field potential recordings. Male Wistar rat offspring (50 days of age) were decapitated under diethyl ether anaesthesia and whole brains were rapidly removed and placed