ORIGINAL ARTICLE

The Endocannabinoid-CB Receptor System: Importance for development and in pediatric disease

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Abstract

Endogenous cannabinoids (endocannabinoids) and their cannabinoid CB1 and CB2 receptors, are present from the early stages of gestation and play a number of vital roles for the developing organism. Although most of these data are collected from animal studies, a role for cannabinoid receptors in the developing human brain has been suggested, based on the detection of "atypically" distributed CB1 receptors in several neural pathways of the fetal brain. In addition, a role for the endocannabinoid system for the human infant is likely, since the endocannabinoid 2-arachidonoyl glycerol has been detected in human milk.

Animal research indicates that the Endocannabinoid-CB1 Receptor ('ECBR') system fulfills a number of roles in the developing organism: 1. embryonal implantantion (requires a temporary and localized reduction in anandamide); 2. in neural development (by the transient presence of CB1 receptors in white matter areas of the nervous system); 3. as a neuroprotectant (anandamide protects the developing brain from trauma-induced neuronal loss); 4. in the initiation of suckling in the newborn (where activation of the CB1 receptors in the neonatal brain is critical for survival). 5. In addition, subtle but definite deficiencies have been described in memory, motor and addictive behaviors and in higher cognitive ('executive') function in the human offspring as result of prenatal exposure to marihuana. Therefore, the endocanabinoid-CB1 receptor system may play a role in the development of structures which control these functions, including the nigrostriatal pathway and the prefrontal cortex.

From the multitude of roles of the endocannabinoids and their receptors in the developing organism, there are two distinct stages of development, during which proper functioning of the endocannabinoid system seems to be critical for survival: embryonal implantation and neonatal milk sucking. We propose that a dysfunctional Endocannabinoid-CB1 Receptor system in infants with growth failure resulting from an inability to ingest food, may resolve the enigma of "non-organic failure-to-thrive" (NOFTT).

Developmental observations suggest further that CB1 receptors develop only gradually during the postnatal period, which correlates with an insensitivity to

the psychoactive effects of cannabinoid treatment in the young organism. Therefore, it is suggested that children may respond positively to medicinal applications of cannabinoids without undesirable central effects. Excellent clinical results have previously been reported in pediatric oncology and in case studies of children with severe neurological disease or brain trauma. We suggest cannabinoid treatment for children or young adults with cystic fibrosis in order to achieve an improvement of their health condition including improved food intake and reduced inflammatory exacerbations

Introduction

Cannabinoid CB1 receptors in the mature organism, are widely and densely distributed in neural as well as non-neural tissue including brain, reproductive, immune, digestive systems as well as in peripheral neurons [1–5]. CB2 receptors are found in non-neural tissue [5, 6], although their presence on peripheral nerves is possible [7]. The presence of CB1 receptors in the developing organism has been studied more thoroughly than that of CB2 receptors.

Cannabinoid CB1 receptors have been described as early as the pre-implantation period in the embryonal mouse [8] and have also been detected around day 11 of gestation [9]. Postnatally, a gradual increase in CB1 receptor mRNA [10], and in the density of CB1 receptors has been measured [11, 12] in whole brain. "Atypical distribution patterns" of CB1 receptors (i.e., a transient presence during development in regions where none are found at adulthood) were detected in white matter regions including the corpus callosum and anterior commissure (connecting neuronal pathways between the left and right hemispheres) between gestational day 21 and postnatal day 5, suggesting a role for endocannabinoids in brain development [13, 14]. Therefore, the varying developmental patterns of cannabinoid CB_1 receptors in different brain regions ([10, 15], are probably due to the transient presence of cannabinoid CB_1 receptors in "atypical" regions during brain development [13].

Strikingly similar developmental patterns of CB1 receptors were found during human pre- and postnatal development. Thus CB1 receptors were detected at week 14 of gestation in the human embryo [16]. In the 20th week of gestation in the human, a selective expression of CB1 receptors was recorded in the limbic area CA of the hippocampus and the basal nuclear group of the amygdala, as compared to a much wider CB1 receptor expression of CB1 mRNA in the adult human brain [17]. A progressive increase in the concentrations of CB1 receptors were found in the frontal cortex, hippocampus, basal ganglia and cerebellum between the fetal period and adulthood [18]. In contrast, high CB1 receptor concentrations were present on several white matter neuronal tracts of the fetus, but which had disappeared by infancy [18]. In the same study it was shown that at all stages of development, the CB1 receptors were functionally active, since the agonist WIN55,212-2 stimulated [^{35}S]GTPγS binding.

Endogenous ligands for the cannabinoid receptors, denoted "endocannabinoids", include thus far, anandamide (arachidonyl ethanol amide, [19]); 2-arachidonoyl glycerol (2AG, [20]), noladin (arachidonyl glyceryl ether [21], the antagonist/partial agonist virodhamine [22] and NADA (N-arachidonoyl-Dopamine, [23]). This newly discovered 'Endocannabinoi-CB Receptor' System, will be denoted here as the 'ECBR' system.

Endocannabinoids have been detected from the fetal period, anandamide at much lower (1000-fold) concentrations than 2-arachidonoyl glycerol [13]. Moreover, the developmental pattern differs between the two endocannabinoids. Thus, whereas concentrations of anandamide gradually increase throughout development until adult levels are reached [15], fetal levels of 2-arachidonoyl glycerol are similar to those in young and in adult brains with a remarkably distinct peak on the first day after birth in rats [15].

In the postnatal hypothalamus, anandamide displays a hardly perceptible rise from day 5 through adulthood, with peak levels immediately before puberty [24].

Thus, the endocannabinoid and their receptors, are abundantly present from the early developmental stages and may therefore be important in the maturation of the nervous system and its functions. Five such functions, are described in the following section.

Functions of the Endocannabinoid-CB Receptor System during prenatal and postnatal development

1. Embryonal Development

CB1 and CB2 receptors are present as early as the pre-implantation mouse embryo, while anandamide binding sites are higher than those in the brain [25]. These observations led to the discovery, that cannabinoids and endocanabinoids arrest the development of 2-cell embryos into blastocytes. Subsequent studies with CB1 and CB2 receptor antagonists indicated that the cannabinoid-induced embryonal growth arrest is mediated by CB1 and not by CB2 receptors [8, 25, 26].

Further, anandamide levels in the uterus are very high; in order for implantation to take place, uterine anandamide levels have to be lowered on the day and on the site of implantation. Failing to do so, prevents implantation of the embryo [26]. Thus the reduction in anandamide is a critical condition for the implantation and hence survival of the embryo.

2. Nervous System Development

Studies on the expression and functionality of the human CB1 receptor in the developing brain have

demonstrated that fetal brain CB1 receptors are functionally active not only in regions which contain CB1 receptors throughout life, such the cerebral cortex and hippocampus, but also in white matter such as the capsula interna and pyramidal tract and in proliferative zones such as the subventricular zone [18]. These observations are consistent with investigations on the developing rat brain, in which "atypical distribution patterns" of CB1 receptors (i.e., a transient presence during development in regions where none are found at adulthood) were detected in white matter regions including the corpus callosum and anterior commissure between gestational day 21 and postnatal day 5. These findings suggest a role for endocannabinoids in brain development [13, 14].

3. Neurotrauma

Similarly to the neuroprotective effects of the endocannabinoid CB1 receptor system in adults [27], activation of CB1 receptor in postnatal rats (7-days old) with WIN55,212, prevented neuronal loss (in a model of acute asphyxia), both immediate and delayed cell death. However, only delayed neurotoxicity was inhibited by the CB1 receptor antagonist SR141716A [28]. Moreover, exogenously applied anandamide reduced ouabain-induced neuronal damage in one and 7 day old rat pups. The CB1 receptor antagonist SR141716A reversed this neuroprotective effect of anandamide only in the 7 day old pups [29]. The latter study also suggested the absence of a tonic neuroprotective activity of the endocannabinoids. Thus neither anandamide nor 2-archidonoyl glycerol were elevated after ouabain-administration. In addition, SR141716A when injected alone, did not enhance the ouabain-induced neuronal damage [29]. In contrast, Hansen et al observed a dramatic increase of anandamide precursors in the infant rat brain after head trauma, which is consistent with a role for anandamide as an endogenous neuroprotectant [27, 31].

4. The newborn period: food intake and survival

The involvement of tetrahydrocannabinol in feeding was shown decades ago [32, 33]; endocannabinoids appear to fulfill a similar role [1, 34]. Endocannabinoids have also been detected in bovine as well as human milk, 2-arachidonoyl glycerol (2AG) in at least 100–1000-fold higher concentrations than anandamide [35, 36].

Is it possible that the high levels of CB1 receptor mRNA and 2-AG which have been observed on the first day of life in structures including the hypothalamic ventromedial nucleus [15] (which is associated with feeding behavior) comprise a major stimulus for the newborn to initiate milk intake?

In a series of studies performed in neonatal mice, we have demonstrated that CB1 receptors are critically important for the initiation of the suckling response. Thus when the CB1 receptor antagonist SR141716A, was injected in newborn mice, milk ingestion and subsequent growth was completely inhibited in most pups (75–100%) and death followed within days after an-

tagonist administration [36]. The antagonist must be administered within 24 h after birth in order to obtain the full effect: Injections on day 2 result in a 50% death rate; SR141716A administration on day 5 has no effect at all on pup growth and survival [33, 37].

Subsequent studies indicated that the catastrophic effect of CB1 receptor blockade is dose dependent and specifically mediated by CB1 receptors. Thus CB2 antagonists did not affect pup suckling and growth, and Δ^9-THC co-administration almost completely reversed the SR141716A-induced growth failure [36]. We have replicated this phenomenon now in three different strains of mice (Sabra, C57BL/6 and ICR).

While these experiments were in progress, CB1 receptor-deficient strains of mice were generated in two different laboratories [38, 39]. In order to resolve the paradox of the existence of mice completely lacking CB1 receptors, with our finding that blockade of CB1 receptors after birth is incompatible with survival, at least for most neonates, we studied early development and milk intake of neonatal CB1$^{-/-}$ pups, provided by Dr. Zimmer's laboratory [37]. Interestingly, CB1 knockout pups did not nurse on the first day of life. However by day 3 of life they had developed normal suckling behavior. Their weight gain though, remained significantly reduced compaired to the C57BL/6 background strain. Further, as expected, the growth curve of CB1 receptor knockout mice, was not affected by neonatal injections of the CB1 antagonist. On the other hand, survival rate and the initiation of the suckling response were significantly inhibited by the CB1 receptor blocker, suggesting the existence of an additional "CB3" receptor, possibly up-regulated in the CB1$^{-/-}$ knockout mice [37].

Recent experiments in our laboratory were designed to further analyze potential physiological/behavioral mediators by which the neonatally administered CB1 receptor antagonist prevents the development of milk ingestion. Thus 2–11 day old pups which had been injected with SR141716A, or with vehicle, within 24 h after birth, were exposed to anesthetized nursing dams. While vehicle-injected pups all located the nipples and nursed from the dam on every testing day, none of the SR141716A-injected pups did so on the day after injection. Only the pups which survived the SR14171A injection, gradually developed the suckling response and suckled like controls by the end of the first week.

Based on the complex relationship between thermoregulation, ultrasonic vocalization [40–42], suckling [43, 44] and maternal behavior [45, 46], we decided to study body temperature and ultrasonic vocalizations, in SR141716A-treated pups throughout postnatal development. Thus we have observed now that the SR141716A-treated pups are hypothermic, while their ultrasonic vocalizations are inhibited (a preliminary report of these data was reported [47]. Present experiments are aimed at delineating the sequence of events induced by the blockade of the CB1 receptor immediately after birth: is a hypothermic pup unable to call his mother to stimulate the suckling response? Or perhaps, does the pup who fails to call his mother

become hypothermic and thus does not have the motor capability to suckle?

Based on data gathered thus far [15, 36, 37], we propose the following model for the initiation of the milk suckling process during the first days postnatally in the mouse: At birth, the 2AG content in the brain is sufficiently high to stimulate the suckling response. Upon milk intake, 2AG from the maternal milk elevates the levels of 2AG in the pup's brain, so that by the second day of life and further on, the milk-derived 2AG stimulates suckling. If endogenous 2AG-induced activation of milk sucking is blocked by the CB1 receptor antagonist, milk is not ingested and hence milk-derived 2AG is not present to stimulate milk sucking on day two of life, and the window to develop a pattern of milk suckling behavior has been closed.

We now propose that the syndrome caused by neonatal blockade of CB1 receptors as a model and perhaps underlying pathology, of "non-organic failure-to-thrive" (NOFTT) condition in infants:

Failure-to-thrive (FTT) is commonly defined as an abnormally low weight and/or height for age [48, 49]. NOFTT is defined as FTT without a known organic cause. Traditionally impaired mother-infant relations were blamed for this condition [50]. We propose that an impaired endocannabinoid-CB1 receptor system may underlie NOFTT. This hypothesis is currently under investigation.

5. Endocannabinoids and the developing prefrontal cortex

Since the 1960-ies, a multitude of studies have attempted the assess potential adverse effects of marijuana use during pregnancy, on the offspring. Although description of the teratogenicity of the cannabis plant and its major psychoactive constituent THC, is beyond the scope of this article, the outcome of such studies have implications for the importance of the ECBR system in development. Thus functions which are not affected by prenatal THC, are unlikely to be under control of the developing ECBR system. Conversely, the development of structures or functions which are significantly altered in the prenatally exposed offspring, are probably regulated at least in part, by this system. Therefore, major trends in the teratogenicity research of cannabinoids will be reviewed briefly.

In animal studies, a multitude of behavioral, hormonal and neurochemical sequelae of prenatal Δ^9-THC exposure have been reported, mainly between the 1960-ies-1980-ies. Taken together, the majority of these early investigations have reported somatic as well as functional impairments, immediately after birth and/or in later life (see [51–53]. It seems however, that confounding variables such as impaired feeding patterns of the cannabinoid-exposed dams [54, 55] may have been responsible for many of these prenatal Δ^9-THC-induced effects.

Recent studies have focused on more specific deficits in the prenatally exposed offspring. For example, prenatal and postnatal exposure to THC interfered with normal dopamine-dependent motor functions and the hypothalamic-pituitary-adrenal stress axis [56–59]. Further, prenatal Δ^9-THC facilitated morphine self administration in the adult female rat offspring, while a number of brain areas including the prefrontal cortex, amygdala and hippocampus displayed altered concentrations of mu-opioid receptors [60]. Memory retention in the adult offspring in a passive avoidance task was disrupted by prenatal exposure to the synthetic cannabinoid WIN55,212. The memory impairment was correlated with a shortening of long-term potentiation and a reduction in extracellular glutamate in the hippocampus [61]. In an elegantly designed prospective study of the children on marihuana smoking mothers, Fried and colleagues have specifically pointed at a subtle but significant impairment of higher cognitive ('executive') functioning, which is ascribed to the prefrontal cortex. This deficiency only becomes apparent from the age of four, since at this age the developing prefrontal cortex becomes functionally expressed [52, 62, 63].

Since the identification of the endocannabinoids (see [1]), prenatal exposure studies have investigated the effects of manipulating the endogenous system, often comparing the results with those from administration of exogenous cannabinoids. These experiments comprise an important extension of the pre- and perinatal studies, because they represent a more physiological manipulation of the ECBR system. Thus, when anandamide, (or Δ^9-THC) was administered daily at low doses (0.02 mg/kg) to pregnant rats, during the last week of pregnancy, only transient, mainly inhibitory, effects on a number of reproductively-relevant hormones were detected. The effects had disappeared within days after birth [64]. Interestingly, Fried and colleagues did not detect changes in pubertal milestones in the children of marijuana smoking mothers [53].

We have found decreased inflammatory response in mice which had been prenatally exposed to anandamide [51].

We [51] have performed experiments on the adult offspring of mice injected daily with anandamide (or Δ^9-THC) during the last week of gestation, with the aim to detect changes specific to the endocannabinoid CB1 receptor system *per se*. Thus when these offspring were observed in the "tetrad" (a series of 4 *in vivo* assays for cannabioid-mediated, or cannabinoid-like effects), they performed similarly to naïve animals which had been injected acutely with 5–10 mg/kg THC [65]. In other words, the prenatally exposed offspring displayed a permanent, moderate "high". Consistent with these data, we also found a higher concentration of CB1 receptors in the forebrain of anandamide, and especially, of the Δ^9-THC-exposed offspring [65]. These data are consistent with an overactive ECBR system and perhaps, a greater vulnerability to the addictive potential of cannabis or other drugs [60].

In a recent study, Viggiano and colleagues [66] induced an elevation of endogenous anandamide levels by administering the reuptake inhibitor AM-404 to pregnant "NHE" rats, an animal model for ADHD

("attention deficit hyperactivity disorder). Thus they reduced certain aspects of hyperactivity and the hypertrophicity of the mesocorticolimbic dopamine system.

Taken together, prenatal manipulation of the endocannabinoid CB1 receptor system, whether by exogenous or endogenous agents, produces long-term effects in the offspring. These include disruptions of memory, addictive and motor behaviors and higher cognitive 'executive' functioning of the prefrontal cortex. Further investigations into the role of the ECBR system in the development of these functions and their neurochemical/anatomical underpinnings, seem therefore warranted.

Cannabinoids as therapy in childhood

The gradual postnatal increase of anandamide and its CB1 receptors [10–12, 15], is accompanied by a gradual maturing response to the psychoactive potential of delta-9-tetrahydrocannabinol (Δ^9-THC) or anandamide in postnatal mice between birth and weaning [67] .

This observation has important implications for cannabinoid therapy in children, since psychoactive side effects may be expected to be minor when treated with cannabinoids at a young age. Indeed, very high doses of Δ^8-THC (approximately 0.64 mg/kg/treatment) were given to children between the ages of 3 and 13 years who were undergoing chemotherapy for the treatment of various hematologic cancers, over long periods of time (up to 114 treatments, based on 4 treatments/24 hr during the days of chemotherapy). The anti-emetic effects were impressive, whereas the side effects were minimal [68]. In a preliminary report [69, 70] eight children (ages 3–14 years) with a variety of severe neurological diseases or damage, were treated with Δ^9-THC (0.04–0.12 mg/kg/day). Significant improvements in behavioral parameters including reduced spasticity, improved dystonia increased interest in the surroundings and anti-epileptic activity were reported without notable adverse effects. The same study reported the case of an 11 year-old girl who suffered a spinal contusion with total paraplegia and a frontal skull fracture following a traffic accident, who was deemed to suffer from posttraumatic disorder with lack of appetite and mood disturbance. The daily THC administration improved appetite as well as mood. Interestingly, this clinical observations is in agreement with two recent animals studies which suggest that the endocannabinoid CB1 receptor system is involved in posttraumatic forgetting [71, 72].

It is not clear, why, in the first study, the anti-emetic effects were achieved (presumably via the *area postrema*) and in the second, positive neurological benefit was derived in the absence of adverse psychological effects. Is it possible that a differential CB1 receptor distribution appears during development, or that certain brain pathways mature at different rates? Clearly, further animal experiments and clinical investigations

of cannabinoid treatment in the developing organism, are warranted.

Cystic Fibrosis is the most frequently occurring recessively hereditary disease affecting 1 in 2500 newborns [73]. In a previous publication [74], we have suggested that a deficient ECBR system may underlie at least some of the symptoms of Cystic Fibrosis, such as malnutrition, gastrointestinal problems, inflammatory exacerbations, and fatty acid imbalance. Therefore, treatment with cannabinoids or drugs which target the ECBR system, such as inhibitors of endocannabinoid reuptake and/or degradation, or CB1 or CB2 receptor agonist, may benefit children (and young adults) afflicted with Cystic Fibrosis.

Conclusions

The endocannabinoids and their receptors (CB1, CB2 and the putative CB3 receptor) [37, 75], fulfill a multitude of physiological functions, including immunological, neurological, psychiatric, cardiovascular. However, from the knowledge accumulated until now, it appears that only in the developing mechanism, at two specific stages, proper functioning of the endocannabinoid-CB receptor system is acutely critical for survival: implantation of the embryo and the initiation of suckling in the newborn.

Interestingly, opposite requirements seem to be involved in each of these processes: a reduction of anandamide is necessary for implantation to take place [8], while the initiation of suckling requires the activation of CB1 receptors, presumably by the presence of high levels of 2AG [33, 36, 37].

Psychoactive side effects of cannabinoid treatment seem to be absent or much reduced in children possibly due to low concentrations of CB1 receptors. Further understanding of the underlying mechanisms will hopefully lead to the development of cannabinoid-based therapeutic strategies for the treatment of disorders including infant "failure-to-thrive" and Cystic Fibrosis.

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