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Ontogenetic development of cannabinoid receptor expression and signal transduction functionality in the human brain

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Abstract

Previous evidence suggests that the endogenous cannabinoid system emerges relatively early during brain development in the rat. However, the pre- and postnatal pattern of appearance of CB_1 cannabinoid receptors in humans has not been analysed in detail. Furthermore, there is a complete lack of information about the functional ability of these proteins to activate signal transduction mechanisms during human development. In the present study we have explored CB_1 receptor expression throughout the different areas of the developing human brain by [^{3}H]CP55 940 autoradiography. We have also assessed CB_1 functional coupling to G proteins during brain development by agonist-stimulated [^{35}S]GTPγS autoradiography in the same cases. Our results indicate a significant density of cannabinoid receptors at 19 weeks' gestation in the same areas that contain these receptors in the adult human brain. Autoradiographic levels of CB_1 receptors in these structures seem to increase progressively from early prenatal stages to adulthood. Interestingly, high densities of cannabinoid receptors have also been detected during prenatal development in fibre-enriched areas that are practically devoid of them in the adult brain. In parallel with these data, we have found that brain cannabinoid receptors are functionally coupled to signal transduction mechanisms from early prenatal stages. This early pattern of expression of functionally active cannabinoid receptors, along with the transient and atypical localization of these proteins in white matter areas during the prenatal stages, suggest an specific role of the endocannabinoid system in the events related to human neural development.

Introduction

Cannabis sativa or marijuana preparations exert widespread and complex effects on higher cognitive functions (emotional changes, enhancement of the senses, impairment of short-term memory), most of which can be attributed to delta-9-tetrahydrocannabinol (Δ^9-THC). In the past decade, the existence of an endogenous cannabinoid system in the brain, as well as in the periphery, has been strongly supported by biochemical, physiological and pharmacological studies, and several endogenous cannabinoid compounds have been isolated (Devane *et al*., 1992; Mechoulam *et al*., 1995; Hanus *et al*., 2001). It has been proposed that many pharmacological effects of cannabimimetic compounds are a result of an interaction with specific cannabinoid (CB) receptors. The structure and biochemical correlates of these receptors are known in detail, and the existence of two different subtypes, CB_1 and CB_2, has been proposed (Devane *et al*., 1988; Matsuda *et al*., 1990; Munro *et al*., 1993). Agonist stimulation of these CB_1 and CB_2 receptors activates several transduction pathways via the $G_{i/o}$ family of G proteins (see Howlett *et al*., 2002 for review).

From the point of view of the anatomical distribution in the central nervous system, autoradiographic and *in situ* hybridization studies in rodents and humans have revealed an important presence of CB_1 receptors in areas such as cerebral cortex, hippocampus, basal ganglia and cerebellum (Herkenham *et al*., 1991; Mailleux & Vanderhaeghen, 1992a; Glass *et al*., 1997).

Different studies support a role for the endogenous cannabinoid system in brain development and maturation. Prenatal exposure to cannabinoids has been shown to modify the maturation process of several neurotransmitter systems, including dopamine, serotonin and opioids (Fernández-Ruiz *et al*., 1999). Furthermore, blockade of CB_1 receptors in newborn mice results in the interruption of suckling behaviour, with subsequent inhibition of neonatal growth (Fride *et al*., 2001). By contrast, some data suggest that prenatal exposure to marijuana could result in a certain impairment of human foetal development (Fried & Smith, 2001), although restricted to a few executive functions. Therefore, it is of clear interest to analyse the pattern of appearance and functional properties of brain CB1 receptors during development. In this regard, the ontogeny of cannabinoid receptors in the rat brain is well known and has been characterized (Romero *et al*., 1997; Berrendero *et al*., 1998, 1999). Cannabinoid receptor binding has been reported to be detectable in developing rat brain even 1 week before the end of gestation. In addition, CB_1 receptors seem to be already functionally coupled to $G_{i/o}$ proteins at these early stages of rat brain maturation, as measured by [^{35}S]GTPγS autoradiography (Berrendero *et al*., 1998).

Regarding humans, data about the appearance and localization of these receptors in the developing brain is still very limited, as only three studies have very partially addressed this issue (Mailleux & Vanderhaeghen, 1992b; Glass *et al*., 1997; Biegon & Kerman, 2001). This lack of knowledge is particularly evident with regard to the late gestational period (third trimester) and the first years of life.

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Furthermore, there is no available information on the transsignalling functionality of cannabinoid receptors during human development.

We here report on CB_1 cannabinoid receptor levels and distribution in post-mortem brain samples of a series of foetuses/neonates and infants, in comparison to adult brains. Moreover, this study assesses for the first time the functionality of cannabinoid receptors during pre- and postnatal development in the human brain.

Materials and methods

Tissues

Human brains were obtained from six foetuses/newborns (three male and three female, age 19–40 weeks' gestation, post-mortem delay 11–38 h at 4 °C, freezing storage period 1–77 months) and five infants and children (five male, age 3–96 months, post-mortem delay 18–24 h at 4 °C, freezing storage period at −25 °C 1–104 months). Tissue from six adults (three male and three female, age 22–74 years, post-mortem delay 8–32 h at 4 °C, freezing storage period at −25 °C 8–27 months) without a reported history of neurological or psychiatric disease were used for comparison. These subjects showed a negative test on the toxicological screening for psychotropic drugs and alcohol. The characteristics of the foetal/neonatal, infant and adult cases included in the study are summarized in Table 1.

The brains were removed at autopsy at the Service of Pathology, University Hospital 'Marqués de Valdecilla', and the tissue collection was approved by the Ethical Committee of this Forensic Institute for post-mortem human studies. Because of the difficulty in obtaining foetal/neonatal and infant brain tissues, only a limited number of tissue blocks (frontal cortex, hippocampus, basal ganglia and cerebellum) could be used for analysis, and not all regions analysed were available from all cases. These blocks were promptly dissected and stored at −70 °C. Consecutive tissue sections were cut at −25 °C using a microtome-cryostat, mounted on gelatinized slides and stored at −25 °C until assay.

Cannabinoid receptor autoradiography

Cannabinoid receptor autoradiography was carried out by incubation of consecutive 15-μm-thick sections in the presence of the cannabinoid agonist [^{3}H]CP55 940 (Dupont/NEN; specific activity 125 Ci/mmol). The incubation procedure was based on the method described by Glass *et al.* (1997), with modifications. Sections were incubated for 2 h at 37 °C with 3 nM [^{3}H]CP55 940 in 50 mM Tris-HCl buffer (pH 7.4) containing 5% BSA. Nonspecific binding was determined in the presence of 10 μM WIN55,212–2 (RBI, Natick, MA, USA). Following the incubation, the sections were washed twice for 2 h at 4 °C, in 50 mM Tris-HCl buffer (pH 7.4) with 1% BSA, and then dipped briefly in distilled water. Finally, the sections were dried on a cold air stream.

Autoradiograms were generated by apposing the labelled tissues to tritium-sensitive films ([^{3}H]-Hyperfilm, Amersham, Buckinghamshire, UK) together with [^{3}H] polymer standards (Amersham microscales). The films were developed after a 15-day exposure at 4 °C. After the scanning of the films, the autoradiograms were analysed as described by Unnerstall *et al.* (1982), using a computerized image analysis system (NIH-IMAGE program, Bethesda, MD, USA). Cannabinoid receptor autoradiographic densities are expressed in fmol/mg tissue equivalent (fmol/mg t.e.), as mean ± SEM.

[^{35}S]GTPγS autoradiography

[^{35}S]GTPγS binding to brain slices was performed according to the protocol described by Sim *et al.* (1996), with several modifications (Rodríguez-Puertas *et al.*, 2000). Sections (20 μm) were incubated for 30 min at 25 °C in a buffer containing 50 mM Tris-HCl, 0.2 mM EGTA, 3 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT, 2 mM GDP and 0.5% bovine serum albumin (BSA) (pH 7.7). Nonspecific binding of the radioligand was determined by isotope dilution in the presence of 10 μM GTPγS. The cannabinoid agonist-stimulated binding was measured under the same conditions in the presence of 100 μM WIN55,212–2. The specifity of the CB_1 receptor mediated-stimulation was verified by coincubation with 10 μM SR141716A (kindly supplied by Sanofi Reserche, Montpellier, France). After the incubation, the slides were washed twice for 15 min at 4 °C in cold 50 mM Tris-HCl buffer (pH 7.4), and dried under a cold air stream. Sections were then exposed to β radiation-sensitive films (Hyperfilm β-max, Amersham, UK) together with [^{14}C] polymer standards (Amersham microscales) for 48 h at 4 °C. The films were developed after a 2-day exposure at 4 °C. After scanning of the films, the autoradiograms were analysed using a

TABLE 1. Sources of foetal/neonatal, infant and adult brain tissue

Case	Gender	Age*	Post-mortem delay (hours at 4 °C)	Freezing storage period (months at −25 °C)	Cause of death
Foetal/neonatal cases					
A	F	19	ND	3	Intrauterine death
B	F	22	17	36	Prenatal distress
C	M	22	17	52	Prenatal distress
D	M	26	38	1	Prenatal distress
E	M	35	3/4	60	Intrauterine death
F	F	40	11	77	Prenatal distress
Infant/children cases					
A	M	3	ND	62	Asphyxia
B	M	8	23	76	Motor vehicle accident
C	M	19	18	104	Drowning
D	M	48	25	62	Motor vehicle accident
E	M	96	24	74	Fire weapon accident
Adult cases					
A	M	22	14	18	Motor vehicle accident
B	F	50	32	13	Motor vehicle accident
C	M	41	20	27	Motor vehicle accident
D	F	74	21	10	Motor vehicle accident
E	F	73	8	8	Neoplasia
F	M	30	24	19	Motor vehicle accident

M, male; F, female; ND, not detectable. *Weeks of gestational age for the foetal/neonatal cases; months of life for the infants/children and years for the adult cases.

computerized image analysis system (NIH-IMAGE program, Bethesda, MD, USA). WIN55,212–2 stimulation values are expressed in percentage over basal activity, as mean ± SEM.

Results

Cannabinoid receptor distribution in the human brain during development

The distribution of [^{3}H]CP55 940 binding sites in the human brain during development is analysed in detail below. We have arbitrarily designated areas with 'very high' density of cannabinoid receptors as those presenting maximal binding densities above 50 fmol/mg tissue. Densities of between 30 and 50 fmol/mg tissue were considered 'high', whereas those between 20 and 30 fmol/mg tissue were 'intermediate'. Finally, those areas with binding densities below 20 fmol/mg tissue were considered as containing 'low' levels of cannabinoid receptors. The values of specific [^{3}H]CP55 940 binding for some selected areas with receptor levels consistently measurable are listed in Table 2. The anatomical distribution of these receptors is further illustrated in Figs 1 and 3.

Frontal cortex

In the foetal/neonatal and infant/children brains the density of [^{3}H]CP55 940 binding sites ranged from intermediate to high, with a predominant presence over the internal layers. The frontal cortex of the adult cases contained very high densities of cannabinoid receptors.

Hippocampus

High densities of [^{3}H]CP55 940 binding sites were observed during the development in the hippocampal formation (CA1 field; Fig. 1) of the different groups of age studied. A clear tendency to the increase in the density of cannabinoid receptors was observed from the foetal period to the adulthood.

Basal ganglia

In the putamen, a progressive increase in the number of [^{3}H]CP55 940 binding sites along the ontogenetic development was observed from the foetal/neonatal to the adult brain. In the globus pallidus lateralis, the density of cannabinoid receptors was intermediate in the infant and childrens' brains, but very high at the adult stage.

Cerebellum

High or very high densities of cannabinoid receptors were observed in the cerebellar cortex (molecular layer) of infant/children and adult cases, whereas the number of cannabinoid binding sites found in the foetal/neonatal cerebellum was intermediate.

White matter areas

The white matter areas of the infant/children and adult brains analysed were completely devoid of [^{3}H]CP55 940 binding sites. In contrast, high densities of cannabinoid receptors were observed over the capsula interna in three foetal cases, all of them below the 30th week of gestation. Furthermore, very high densities were found in the pyramidal tract of the 19-week-old foetus brain (Fig. 3), and in the brachium conjunctivum of the 26-week-old foetal brain.

Proliferative zones

Very high levels of cannabinoid receptors were observed at the neocortical subventricular area in two foetal cases of 19 and 22 weeks' gestation.

Cannabinoid receptor activation of signal transduction mechanisms in the human brain during development

Activation of cannabinoid receptors with the agonist WIN55,212–2 increased [^{35}S]GTPγS binding in all areas that contained measurable [^{3}H]CP55 940 binding levels in the developing human brain. We have arbitrarily designated areas with 'very high' values of stimulation as those presenting percentages of maximal stimulation above 400%. Values of between 300 and 400% were considered as 'high' and those between 200 and 300% as 'intermediate', whereas levels of stimulation between 100 and 200% were included in the 'low' range. Stimulation values below 100% were considered as 'very low'.

In all cases, the antagonist SR141716A completely reversed WIN55,212–2-induced stimulation of [^{35}S]GTPγS binding, indicating the specific involvement of CB_1 cannabinoid receptors in this effect. WIN55,212–2-induced stimulation values for the selected areas with measurable cannabinoid receptor levels are listed in Table 3. The anatomical distribution of the cannabinoid agonist activation of [^{35}S]GTPγS binding in the developing human brain is further illustrated in Figs 2 and 3.

TABLE 2. Cannabinoid receptor binding measured by [^{3}H]CP55940 autoradiography in the developing human brain

	Cannabinoid receptor density (fmol/mg t.e.)*					
	Foetal/neonatal	(*n*)	Infants/children	(*n*)	Adults	(*n*)
Frontal cortex (internal layers)	28.8 ± 13.5	5	45.5 ± 10.6	5	57.1 ± 10.5	6
Hippocampus						
CA1	34.7 ± 10.2	5	38.7 ± 12.1	5	91.2 ± 21.5	5
Dentate gyrus	n.d.	–	26.1 ± 18.3	2	102.6 ± 18.2	4
Basal ganglia						
Putamen	36.7 ± 21.2	5	52.6 ± 21.3	4	99.5 ± 11.2	6
Globus pallidus lateralis	n.d.	–	26.1 ± 7.3	4	139.3 ± 32.6	4
Cerebellum (molecular layer)	22.7 ± 6.8	5	48.7 ± 19.2	4	71.4 ± 14.4	3
White matter areas						
Capsula interna	36.1 ± 14.3	3	ND	–	ND	–
Pyramidal tract	95.3	1	ND	–	ND	–
Brachium conjunctivum	138.6	1	ND	–	ND	–
Subventricular geminative zones	66.9 ± 25.6	2	ND	–	ND	–

*Values are expressed as B_{max} densities (fmol/mg tissue, mean ± SEM). ND, not detectable; n.d., not determined.

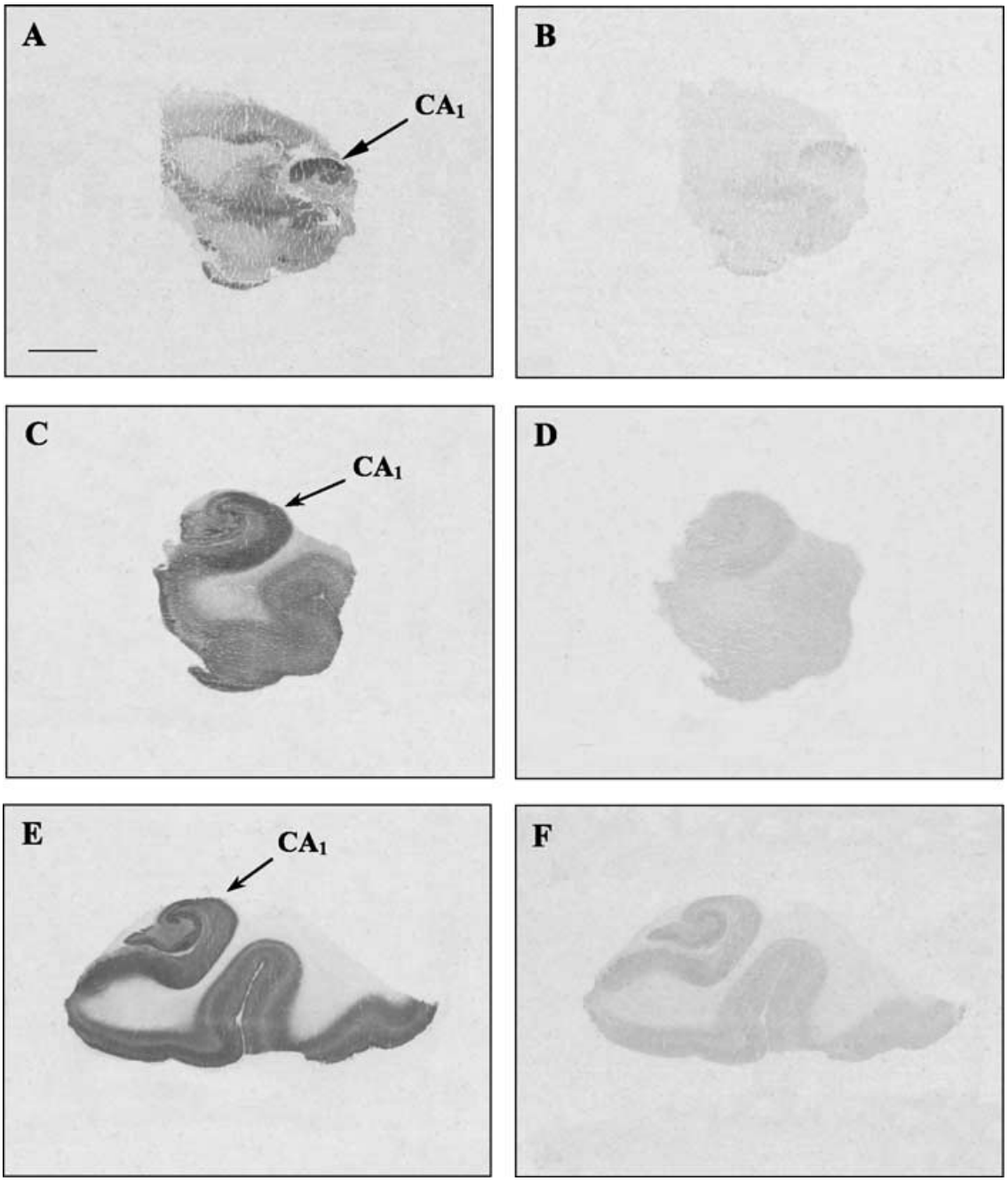

FIG. 1. Autoradiographic images corresponding to [^{3}H]CP55 940 binding in post-mortem human hippocampal sections obtained from a 19 weeks' gestation foetal brain (case A; pictures A and B), from a 8-month-old infant brain (case B; pictures C and D), and from and adult brain (case E; pictures E and F). (A, C and E) Total binding. (B, D and F) Nonspecific binding. Note the high levels of cannabinoid receptors in the CA_1 field of the hippocampus at 19 weeks' gestation. Scale bar, 5 mm.

Frontal cortex

In this area, intermediate levels of WIN55,212–2-induced stimulation were observed both in the foetal/neonatal and adult brains. By contrast, the frontal cortex of the infants and children showed low values of stimulation (Fig. 2).

Hippocampus

High levels of cannabinoid agonist-induced activation of [^{35}S]GTPγS binding were measured in the CA_1 field of the fetal/neonatal and adult brains, whereas the infants/children exhibited low values of stimulation in this anatomical region. WIN55,212–2-induced stimulation in the dentate gyrus of both infant/children and adult brains ranged from low to intermediate values.

Basal ganglia

Low to intermediate levels of cannabinoid agonist-induced stimulation of [^{35}S]GTPγS binding were observed in the putamen of both infant/children and adult brains. In contrast, very high levels of stimulation were detected in the putamen during the fetal and neonatal period. The globus pallidus lateralis showed values of WIN55,212–2-induced stimulation ranging from intermediate levels in the infants and children to very high levels in the adult cases.

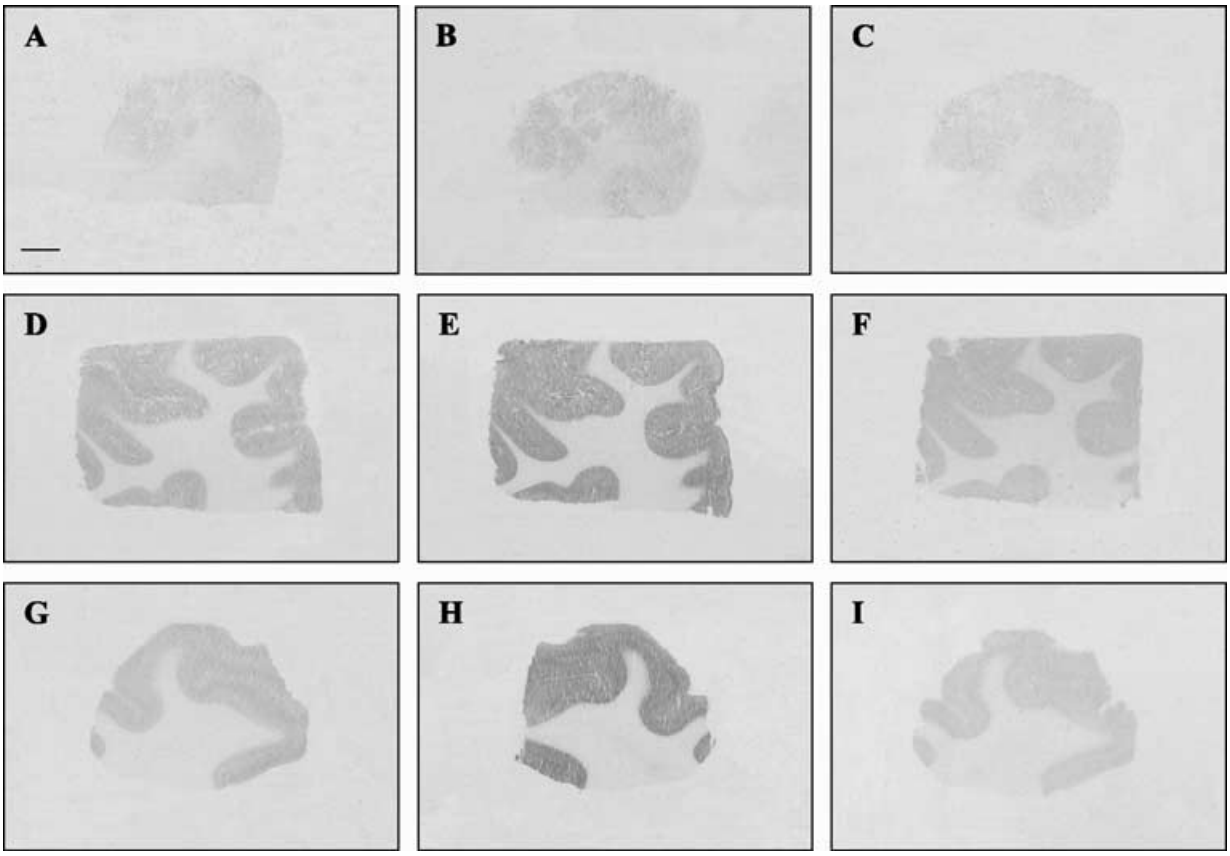

FIG. 2. Autoradiographic images corresponding to [^{35}S]GTPγS binding in post-mortem human frontal cortex sections from a 40 weeks' gestation foetus (case F; pictures A–C), from a 4-year-old-child (case D; pictures D–F) and from an adult brain (case A; pictures G–I). (A, D and G) Basal binding. (B, E and H) Binding in the presence of 100 μM WIN55,212–2. (C, F and I) Binding in the presence of 100 μM WIN55,212–2 and 10 μM SR141716A. Scale bar, 5 mm.

Cerebellum

A clear tendency to an increase in WIN55,212–2-induced activation of cannabinoid receptors was observed in the human cerebellar cortex during brain development; cannabinoid agonist stimulation levels ranged from very low values in foetal and neonatal brains to very high values in adult cases.

White matter areas

In the white matter areas of the infant/children and adult brains analysed no measurable levels of WIN55,212–2-evoked stimulation were detected. In contrast, intermediate levels of stimulation were found in the capsula interna of three foetal cases (19, 22 and 26 weeks' gestation). Furthermore, the 19- and the 26-week-old foetal brains also showed significant values of cannabinoid activation in the pyramidal tract and the brachium conjunctivum, respectively (Fig. 3).

Proliferative zones

Very high levels of WIN55,212–2-induced stimulation were found over the neocortical subventricular zone in two cases of 19 and 22 weeks' gestation.

Discussion

The presence of cannabinoid receptors, as well as their degree of functional coupling during ontogeny, has been explored extensively in the rat brain. However, data regarding CB_1 protein expression in the developing human brain are still scarce, particularly regarding the late gestational period and the first years of life. In addition, there is a complete lack of information about cannabinoid receptor ability to activate signal transduction mechanisms during the pre- and postnatal maturation of the human brain. This study analyses cannabinoid receptor binding levels in immature human brain including a wide range of developmental stages (from 19 weeks' gestation to 8 years of life), and addresses in parallel the G-protein-coupling ability of these receptors in the same foetal/neonatal and infant cases, in comparison to adult brain.

The anatomical pattern of distribution of CB_1 receptors found in this study (high densities in cortical layers, CA1 field of the hippocampus, basal ganglia, cerebellar cortex) is, in general terms, in good correlation with that reported for the adult human brain, as suggested by previous studies performed on narrower bands of developmental age (Mailleux & Vanderhaeghen, 1992b; Glass *et al*., 1997; Biegon & Kerman, 2001). This autoradiographic study confirms that, in general agreement with the temporal pattern of appearance of CB_1 receptors reported for rat brain (Romero *et al*., 1997; Berrendero *et al*., 1999), significant levels of these proteins are present in the foetal human brain from relatively early stages of development to the late gestational period. This early timing of expression of cannabinoid receptors in the developing human brain has been reported recently by Biegon & Kerman (2001). These authors found levels of CB_1 receptors in the foetal human brain as early as 14 weeks' gestation, although the autoradiographic densities of these sites through the second trimester appeared to be very low in comparison to those found in adults, with the exception of the globus pallidus. In slight contrast, our data indicate that other anatomical regions of the developing human brain at 19–22

TABLE 3. WIN55212-2-stimulated [^{35}S]GTPγS binding measured by autoradiography in the developing human brain

	WIN55212-2-stimulated specific [^{35}S]GTPγS binding (%)*					
	Foetal/neonatal	(*n*)	Infant/children	(*n*)	Adults	(*n*)
Frontal cortex (internal layers)	278 ± 61	5	127 ± 26	5	265 ± 63	6
Hippocampus						
CA_1	356 ± 165	5	113 ± 46	3	380 ± 96	5
Dentate gyrus	n.d.	–	194	1	276 ± 51	5
Basal ganglia						
Putamen	1496 ± 601	5	160 ± 35	4	290 ± 121	6
Globus pallidus lateralis	n.d.	–	221 ± 106	4	963 ± 220	4
Cerebellum (molecular layer)	67 ± 46	3	592 ± 217	5	555 ± 97	4
White matter areas						
Capsula blanca	177 ± 80	3	ND	–	ND	–
Pyramidal tract	1954	1	ND	–	ND	–
Brachium conjunctivum	167	1	ND	–	ND	–
Subventricular proliferative zones	844 ± 687	2	ND	–	ND	–

*Values are expressed as percentages of basal binding (mean ± SEM). ND, not detectable; n.d., not determined.

weeks of gestation (putamen, hippocampal CA1 field) also contain significant levels of cannabinoid receptors. It is difficult to suggest an explanation for this partial discrepancy, taking into account the differences between both studies: different sources of tissue, gestational ages, experimental procedures, freezing storage period, etc. In this regard, it is noteworthy that our results showing the presence of neonatal cannabinoid receptor densities close to those found at the adult stage are in good agreement with the results reported by Glass *et al*. (1997). This prenatal pattern of cannabinoid receptor appearance is similar to that reported for other, but not all, neurotransmitter receptors in the human brain. In this sense, serotonin 5-HT_{1A} and dopamine D_2 receptor densities at the 26th week of gestation have been shown to be similar of even higher than those found in adults, whereas the development of 5-$HT_{1B/D}$ receptors in the human brain is mainly postnatal (Del Olmo & Pazos, 2001).

Our results also suggest that CB_1 autoradiographic densities increase progressively from early gestational stages to adulthood in most anatomical regions (frontal cortex, hippocampus, basal ganglia and cerebellum), in agreement with previous studies on cannabinoid receptor expression during rat brain maturation (Berrendero *et al*., 1999). Unfortunately, an exact comparison of cannabinoid receptor expression levels between foetal/neonatal, infant and adult cases is somehow hampered by the existence of marked differences in the freezing storage period between the three groups of cases. Long storage periods at −25 °C are common in autoradiographic studies performed on human brain because it is necessary to keep tissue blocks at this temperature after the first sectioning process. This difficulty is especially evident when working with foetal/neonatal and infant brain samples, as a collection of a reasonable number of cases requires large periods of time. In this regard, it must be taken into account that storage of the tissue blocks at −25 °C exerts a negative influence on neurotransmitter receptor expression and functional coupling in the human brain, as has been shown for muscarinic and monoamine receptors (Kontur *et al*., 1994; Rodríguez-Puertas *et al*., 1996; unpublished data). As the average freezing storage period at −25 °C of the foetal/neonatal and infant brain samples in the present study is notably higher than that calculated for the adult tissues, it is possible that the lower cannabinoid receptor densities reported for these cases in comparison with adults probably reflect not only reduced CB_1 levels during human brain development but also a more marked loss of cannabinoid protein associated with longer storages at −25 °C.

[^{35}S]GTPγS assays performed in brain sections from the same foetal/neonatal, infant and adult cases indicate that the reported cannabinoid protein autoradiographic densities represent functional receptors, as they are coupled to GTP-binding proteins of the $G_{i/o}$ subtype, and therefore are able to activate signal transduction mechanisms. This CB_1 receptor functional coupling to G proteins is evident for all the anatomical regions tested and from the earliest gestational age analysed (19 weeks' gestation). In this regard, our results are in good agreement with the early development of cannabinoid receptor functionality during rat brain maturation, as Berrendero *et al*. (1998) described significant cannabinoid agonist-stimulated [^{35}S]GTPγS binding levels from gestational day 16. The lower degree of [^{35}S]GTPγS stimulation observed in the infant/children group could be because of the longer storage period for these cases. With regard to the very high levels of G protein activation found at the putamen of the foetal/neonatal cases, we do not have a conclusive explanation at the present time. It is noteworthy that Glass *et al*. (1997) reported very high densities of CB_1 receptors in the neonatal putamen.

One of the limitations in the studies involving human post-mortem material is the limited availability of brain tissue (Pazos & Palacios, 1989), that becomes of critical relevance when foetal tissue is used (Del Olmo & Pazos, 2001). In addition, the level of anatomical definition of brain nuclei is somewhat limited in the tissue blocks from the most immature cases. Because of all this, it has not been always possible to perform in parallel, in all tissue blocks, receptor protein and [^{35}S]GTPγS visualization. These factors, together with the previously discussed variability in the time of freezing storage, determine the complexity of the analysis of quantitative data in these kinds of studies.

Evidence from different experimental approaches indicate that endocannabinoids, through the activation of G-protein-linked responses, play a relevant role in the development of the nervous system (Fernández-Ruiz *et al*., 2000). In the rat, prenatal exposure to Δ^9-THC has been shown to modify the maturation of different neurotransmitter systems, including catecholamines and serotonin (Walters & Carr, 1988; Rodriguez de Fonseca *et al*., 1991; Molina-Holgado *et al*., 1996). It results in an increase in the density and functional activity of tyrosine hydroxylase, the key enzyme in the catecholaminergic biosynthetic cycle (Hernández *et al*., 1997). Early prenatal exposure to cannabinoids results in structural abnormalities in the rat

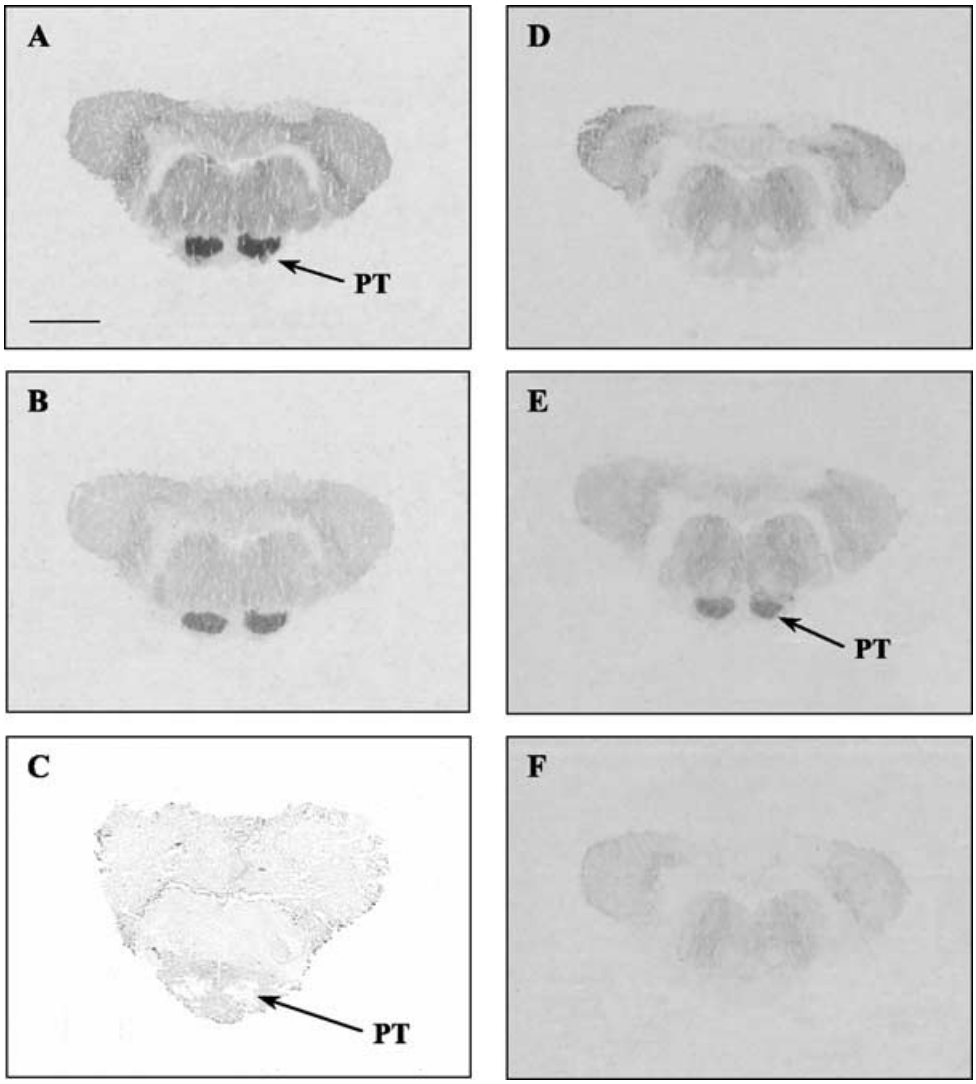

FIG. 3. Autoradiographic images corresponding to [^{3}H]CP55 940 (A and B) and [^{35}S]GTPγS binding (D–F) in post-mortem human brain sections from a foetus of 19 weeks' gestation. (A) Total [^{3}H]CP55 940 binding. (B) Non-specific binding. (C) Cresyl violet staining. (D) Basal [^{35}S]GTPγS binding. (E) [^{35}S]GTPγS binding in the presence of 100 μM WIN55,212–2. (F) [^{35}S]GTPγS binding in the presence of 100 μM WIN55,212–2 and 10 μM SR141716A. PT, pyramidal tract. Scale bar, 5 mm.

foetus (Dalterio, 1986). Furthermore, activation of CB receptors present in cultured foetal nerve cells has a role in the expression of several genes as well as in the regulation of signalling mechanisms (Bouaboula *et al*., 1995; Shivachar *et al*., 1996). In good agreement with that, some data suggest that prenatal exposure to marijuana could affect human foetal development (Fried & Smith, 2001). The present results strongly support cannabinoid involvement in the development of the human nervous system. First, they demonstrate the existence of a significant amount of functionally active CB_1 receptors in the brain at relatively early prenatal stages and second, the presence of CB_1 receptor protein and functionality in the neocortical subventricular proliferative zones in two early foetal cases indicates a role for these receptors in the cell proliferating process.

An interesting finding of our study is the presence of high levels of CB_1 receptors and cannabinoid agonist-induced stimulation of G proteins in white matter areas, in particular fibre tracts, that present very low levels in the adult brain. Thus, the capsula interna presented high levels of both CB_1 receptor density and functionality in three foetal cases of early gestational ages (from 19-26 weeks). Similarly, the pyramidal tract and the brachium conjunctivum also contained elevated densities. As discussed previously, the very limited availability of tissue has not allowed us to confirm this transient appearance of CB_1 receptors for more tracts in all cases analysed. However, the presence of CB_1 receptors (protein, messenger RNA and G protein activation) in white matter areas of the developing rat brain has been well reported and characterized, and their possible localization to non-neuronal (glial) cells has been discussed (Romero *et al*., 1997; Berrendero *et al*., 1998). As suggested for other neurotransmitter receptors (see Del Olmo & Pazos, 2001), the early and transient presence of these receptors could indicate a specific role for the endocannabinoid system in several developmental events, such as metabolic support, cell migration and myelin formation.

In conclusion, our results demonstrate for the first time that high densities of CB_1 receptors, functionally coupled to G proteins are present at early developmental stages throughout the human brain, strongly suggesting their involvement in the events related to neural development.

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Abbreviations

CA, cornus Ammonis; CB, cannabinoid; SEM, standard error of the mean.

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