

Autoradiographic Study of Pre- and Postnatal Distribution of Cannabinoid Receptors in Human Brain¹

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Cannabinoid receptors have been characterized and localized in the brain of several species, including human. The pre- and postnatal distribution of human brain CB1 receptors was investigated using quantitative autoradiography with [³H]CP55,940 as a ligand. Normal fetal brains (*N* = 8, gestational age 14–24 weeks) were obtained from voluntary abortions. Normal (drug and pathology free) adult human brains (*N* = 16, age 18–78) were obtained from the medical examiner's offices in New York City and Jaffa, Israel. Brains were stored frozen at –70°C and sectioned (40 μm) at –15°C. The radioligand (5 nM) was incubated with the sections for 3 h at room temperature. Washed and dried sections were exposed to tritium-sensitive film along with standards for 7–28 days and autoradiograms quantitated using NIH Image software. In the fetal human brain, low densities of THC-displaceable, region-specific binding could be observed as early as 14 weeks gestation. Receptor density increased slowly with gestational age but did not reach adult levels by the end of the second trimester (24 weeks gestation). In addition, the distribution pattern in the fetal brains was markedly different from the adult pattern. The most striking difference was the very low density of binding in the fetal caudate and putamen. In contrast, the globus pallidus pars medialis has almost-adult levels of cannabinoid receptors by 17–18 weeks gestation. The relatively low and regionally selective appearance of cannabinoid receptors in the fetal human brain may explain the relatively mild and selective nature of postnatal neurobehavioral deficits observed in

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INTRODUCTION

Although cannabis-derived drugs have been used for thousands of years, brain receptors and endogenous ligands for cannabinoids are a relatively recent discovery (e.g., Devane *et al.*, 1988, 1992; Howlett *et al.*, 1990; Matsuda *et al.*, 1990; Martin *et al.*, 1999). In adult mammalian brain, autoradiography has revealed high densities of cannabinoid receptors in many brain regions, which presumably subserve the known behavioral and physiological effects of cannabinoids (Herkenham *et al.*, 1990, 1991).

Marijuana is one of the most widely used recreational drugs in our society. Drug dependence is more common in men than in women, peaks in young adults, and goes down steadily with age (Burke *et al.*, 1990). However, there is evidence that a significant percentage of pregnant women, 3–27% according to various reports (e.g., Zuckerman *et al.*, 1985, 1989; Hatch *et al.*, 1986; MacGregor *et al.*, 1990; Lee, 1998), use the drug during pregnancy. THC (the active component of marijuana) crosses the placenta readily in rodents (Idanappan-Heikkila *et al.*, 1969) and primates (Bailey *et al.*, 1987), peaking in fetal blood within 15 min of administration. THC has also been found in the umbilical cord blood at delivery of women who used marijuana regularly during pregnancy (Blackard and Tennes, 1984), which indicates that THC crosses the placenta in humans as well. Interestingly, reports on CNS effects of cannabinoid exposure during pregnancy are contradictory and overall, marijuana appears to have only minor effects on the offspring, in contrast with the effects of other addictive substances that cross the placenta, such as nicotine, alcohol, opiates, and cocaine (e.g., Fried, 1980; Fried and Watkinson, 1990; Zuckerman *et al.*, 1989; Balle *et al.*, 1999). In the present study we have examined the neuroanatomical distribution and developmental pattern of cannabinoid recep-

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tors in brains of normal fetuses and adults by quantitative autoradiography using the specific ligand [³H]CP55,940.

MATERIALS AND METHODS

Brains

Normal fetal brains ($N = 8$, gestational ages 14, 17, 18, 20, 22, and 24 weeks) were obtained from saline-induced voluntary abortions. Normal (drug and pathology free) adult human brains ($N = 16$, age range 18 to 78) were obtained from the medical examiner's offices in New York City and Jaffa, Israel. The brains were frozen in dry ice and stored at -70°C . Both the fetal and the adult brain populations have been previously described in detail (Bar Peled *et al.*, 1991a,b, 1997; Biegon *et al.*, 1988; Gross-Isserof *et al.*, 1990).

Thin (40 μm) sections of one hemisphere, cut in the coronal or sagittal plane, were obtained using a whole-body cryotome (Bright) at -15°C . The sections were thaw mounted onto gelatin-chromalum-coated slides and stored at -20°C at least overnight prior to the autoradiography procedure.

Autoradiography

A 5 nM concentration of [³H]CP55,940 (NEN, sp act 104 Ci/mmol) was incubated with the sections for 3 h at room temperature in a 120 mM Tris-HCl buffer, pH 7.4, containing 5% BSA. The incubation was followed by 2×90 -min washes in the same buffer containing 1% BSA, at 4°C . Nonspecific binding was determined on consecutive sections in the presence of 100 μM unlabeled THC. The washes were followed by a short dip in double-distilled water to remove buffer salts.

Dried sections were exposed to tritium-sensitive film for 1 to 4 weeks, alongside commercial (Amersham) tritium standards. Films were developed manually using Kodak developer and fixer. The sections were stained with cresyl violet for anatomical reference.

Autoradiograms and tritium standards were quantified using a computerized, video camera-based image analysis system and the NIH Image software. Regions were identified in reference to the histologically stained sections and human brain atlases.

RESULTS

In the fetal human brain, low densities of THC-displaceable, region-specific binding could be observed as early as 14 weeks gestation. Receptor density increased slowly with gestational age but did not reach adult levels by the end of the second trimester (24 weeks gestation). In addition, the distribution pattern in the fetal brains was markedly different from the adult pattern. The most striking difference was the

very low, patchy density of binding in the fetal caudate and putamen. The globus pallidus is the only region in fetal brain demonstrating relatively high levels of cannabinoid receptors by 17–18 weeks gestation (Fig. 1). In contrast, the distribution of cannabinoid receptors in the adult human brain is widespread although heterogeneous with high levels of specific binding in many brain regions (Fig. 2). Thus, very high densities were found in the substantia nigra, medial portion of the globus pallidus, and basal ganglia. Moderate densities were found in the cortex and hippocampus. Low densities were observed in most of the thalamus. Cortical binding is laminar, such that a thin high-density band over layer I is followed by lower binding in intermediate layers and high binding in inner cortical layers. Nonspecific binding is relatively low and uniform over gray matter areas of the brain (Fig. 2).

Quantification of fetal receptor levels was possible only in the few regions showing a density consistently higher than nonspecific binding (Table 1). Thus, binding density in both parts of the fetal globus pallidus was approximately half of the mean value in young adults (Table 1). The distribution of binding within the globus pallidus in the fetal brains was similar to that of the adult as well, with the pars medialis demonstrating consistently higher levels than pars lateralis. However, binding in the caudate was patchy and low. Hippocampal binding, although visible, was only slightly above the level of nonspecific binding. Cortical levels of cannabinoid binding in the fetus were higher over inner cortical layers, reminiscent of the adult pattern, but practically nondetectable in the outer cortical layers.

DISCUSSION

In this study of fetal human brains, cannabinoid receptors were detectable as early as 14 weeks gestation. However, receptor levels were very low throughout the second trimester and the only region demonstrating receptor density close to that seen in adults was the globus pallidus. The highest densities of cannabinoid receptors in the adult brain, as previously reported by us and others (Herkenham *et al.*, 1990; Biegon and Kerman, 1995), are associated with elements and outflow regions of the dopaminergic system. The substantia nigra, the major dopamine-producing region, contains the highest density of binding, followed by the medial part of the globus pallidus, the caudate, and the putamen. However, many other brain regions contain moderately high concentrations of radioactivity, including the serotonergic raphe nuclei and the noradrenergic nucleus locus coeruleus. This pattern suggests that cannabinoids may be powerful, though indirect, modulators of dopaminergic activity in the brain. The moderately high densities of binding in the raphe and locus coeruleus suggest that cannabi-

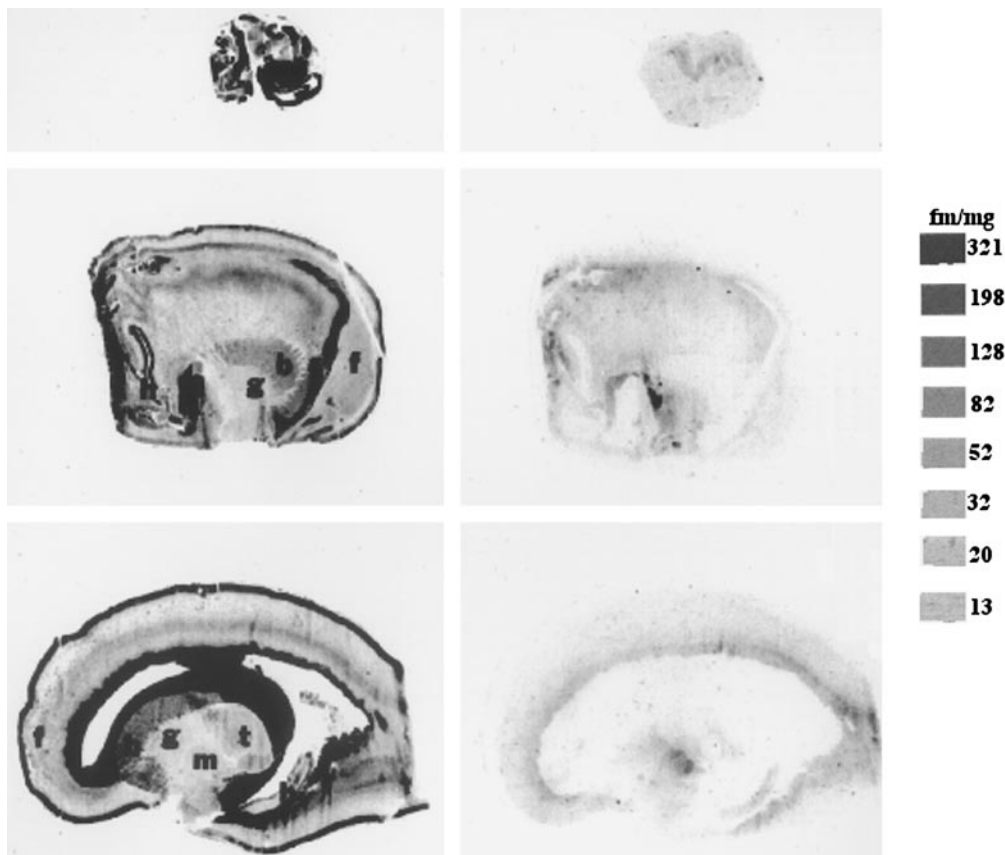


FIG. 1. Distribution of cannabinoid receptors in the second-trimester human fetal brains postmortem. The images on the left are photographs of the cresyl violet sections used to produce the matching autoradiograms on the right. The top row is from gestational age 14 weeks, the middle row is from a 17-week-old fetus, and the bottom row represents a 24-week-old fetus. The sections are in the sagittal plane and were exposed to film, together with a tritium standard scale strip (right) for 28 days. The scale bars (from top to bottom) correspond to [^3H]CP55,940 in fm/mg (calculated from the nCi/mg content of the standards and the specific activity of the ligand used in the experiment). Abbreviations: f, frontal cortex; b, basal ganglia; g, globus pallidus; m, medial part of globus pallidus; h, hippocampus; t, thalamus.

noids may also modulate serotonergic and noradrenergic activity, although probably to a lesser degree than dopaminergic activity.

The developmental results reported here are compatible with recent developmental studies in rodents showing a slow progression of receptor density during prenatal and postnatal development in the rat (Belue *et al.*, 1995; Romero *et al.*, 1997; Berrendero *et al.*, 1999). The single human study reporting high fetal brain levels of cannabinoid receptors (Glass *et al.*, 1997) included only one fetal brain, so it is difficult to speculate on the reasons for the apparent discrepancy. Slow and relatively late development of cannabinoid receptors in the brain may explain the paucity of postnatal developmental effects observed in rats treated perinatally with cannabinoids and in children born to women who have used marijuana during pregnancy. Thus, some researchers have found that prenatal marijuana exposure may affect fetal growth while having no teratogenic, neurobehavioral, or other developmental effects following birth (Tennes *et al.*, 1985; Mirochnik *et al.*, 1997; Balle *et al.*, 1999). Others have found

that offspring born to heavy (>5 joints a week) marijuana users had a significantly higher occurrence of tremors and startles (Fried, 1985; Fried and Mackin, 1987), which also differed in their nature from those of non- and light users. As part of the same study, it has also been reported that the prenatally exposed offspring showed poorer habituation to light stimulus and poorer visual responsiveness at 3, 9, and 30 days of age. These infants also showed a trend toward greater incidence of myopia, strabismus, and abnormal oculomotor functioning (Fried *et al.*, 1987). Furthermore, these infants performed significantly worse than the controls on a number of motor tests (Fried *et al.*, 1987), which, along with the reported trend toward greater irritability, is consistent, though milder in nature, with postnatal opioid withdrawal (Fried and Watkinson, 1988, 1990; Zuckerman *et al.*, 1989).

In rats, chronic cannabinoid administration to adult males and females results in widespread and significant decreases in cannabinoid receptor density (Oviedo *et al.*, 1993; Romero *et al.*, 1998; Breivogel *et al.*, 1999). In contrast, exposure of fetal brains to cannabinoids *in*

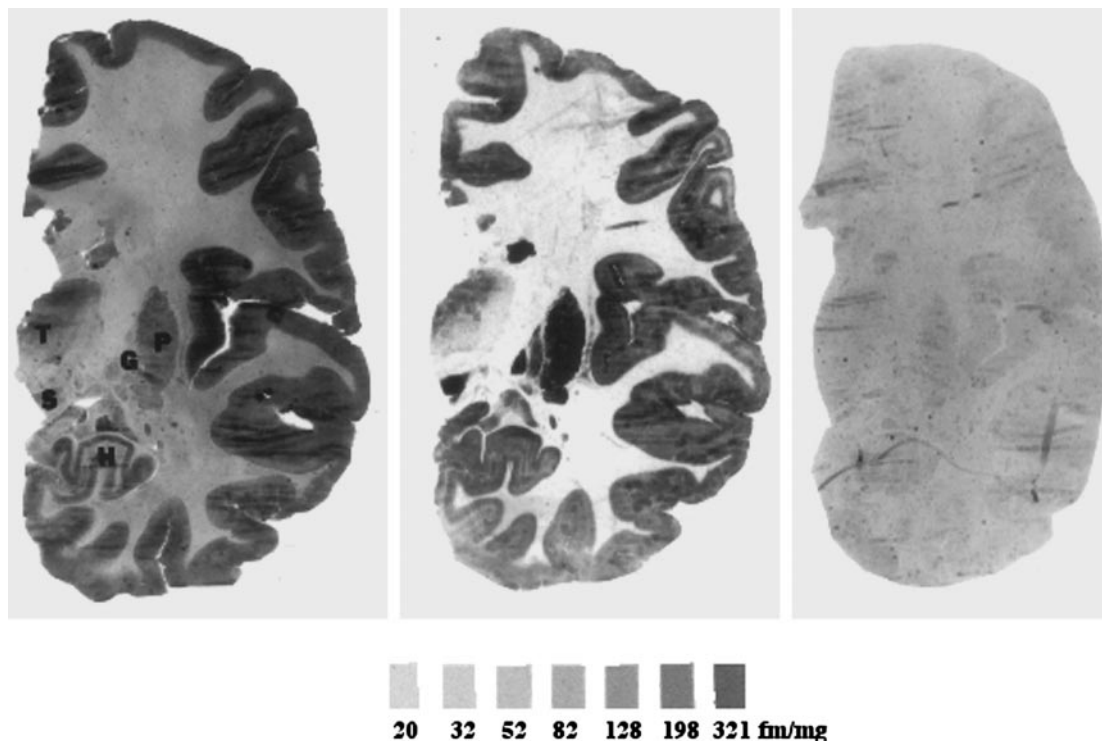


FIG. 2. Distribution of cannabinoid receptors in the adult human brain postmortem. A coronal section of the right hemisphere at the level of the hippocampus is shown. The image on the left is the cresyl violet-stained section used to produce the autoradiogram in the center. The image on the right is an autoradiogram of a consecutive section incubated with excess unlabeled THC representative of nonspecific binding. The sections were exposed to film, together with a tritium standard scale strip (shown at the bottom) for 7 days. At this short exposure time the lowest standard was not visible above film background. The concentrations of ligand corresponding to the bars are the same as in Fig. 1 above. The nonspecific autoradiogram was enhanced so it would be visible; radioactivity levels in those sections were below 20 fm/mg. Abbreviations: T, thalamus; P, putamen; G, globus pallidus (note the higher density of binding in the medial compared to the lateral part of the structure); S, substantia nigra; H, hippocampus.

utero had no effect on adult receptor levels (Garcia Gil *et al.*, 1999). Also in the rat, peak levels of receptors appear between days 30 and 40 of postnatal life (Rodriguez de Fonesca *et al.*, 1993) and aging is associated with region-specific decline in receptor and mRNA levels (e.g., Berrendero *et al.*, 1998), which was especially pronounced in deep cortical layers. We have reported a similar decline in receptor binding in human prefrontal and cingulate cortex (Biegon and Kerman, 1995). Therefore, it appears that although the distribution of

cannabinoid receptors is not identical in rat, monkey, and human (Biegon and Kerman, 1995; Ong and Mackie, 1999), the age-dependent changes are similar in rodents and man.

Further studies are necessary to extend the developmental curve of cannabinoid receptors to infants and children. Such data may explain another apparently paradoxical observation in children given THC as an antiemetic, who do not show the psychotropic profile seen with the same drug in adults (Abrahamov *et al.*, 1995). These young (less than 10-year-old) patients demonstrated a remarkable absence of psychotropic effects. Correspondingly, it was found that behavioral responses to THC develop relatively late in mice (Fride and Mechoulam, 1996). The fact that the antiemetic effect of the drug was apparent in the pediatric patients does not contradict a relative lack of brain CB1 receptors, since the antiemetic properties of cannabinoids are not mediated by the cannabinoid receptor. In fact, antiemetic activity can be elicited with nonpsychoactive synthetic cannabinoids which have an optical configuration opposite to the one favored by the cannabinoid receptor (Feigenbaum *et al.*, 1989a,b).

TABLE 1

Comparison of Cannabinoid Receptor Density in Basal Ganglia of Second-Trimester Fetuses and Young Adults

Brain region	Specific binding, fmol/mg protein	
	Fetal brains	Adult brains
Caudate	77.2 ± 41.4	437 ± 203
Globus pallidus, medial	289.2 ± 114	567 ± 175
Globus pallidus, lateral	140.9 ± 94.0	309 ± 124

Note. Results are means and standard deviations of six fetal and three adult brains.

From the perspective of brain development, it is noteworthy that in consecutive sections from the same brains used for these studies, we found densities of cholinergic muscarinic and 5HT_{1a} receptors that were quite high, often higher than in adults. Subtypes of glutamate transporters were also detected at high levels in the same brains (Bar Peled *et al.*, 1991a,b, 1997). Thus, the late development of cannabinoid receptors is not a common feature of brain neurotransmitter markers. This pattern may actually differentiate between markers that mature in parallel with brain maturation and those that play a specific role (e.g., guidance of growth cones and synapse formation) in brain development. It would appear that cannabinoid receptors belong to the former class, and the relatively low and regionally selective appearance of cannabinoid receptors in the fetal human brain may explain the relatively mild and selective nature of postnatal deficits observed in infants exposed to cannabinoids *in utero*.

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