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Cannabinoid-Induced Alterations in Regional Cerebral Blood Flow in the Rat

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BLOOM, A. S., S. TERSHNER, S. A. FULLER AND E. A. STEIN. *Cannabinoid-induced alterations in regional cerebral blood flow in the rat.* PHARMACOL BIOCHEM BEHAV **57**(4) 625–631, 1997.—A specific receptor for cannabinoids has been characterized at the pharmacological, molecular, and neuroanatomical level. However, less is known of the functional localization in the brain for the behavioral and physiological actions of these drugs. We have examined the effects of Δ^9-tetrahydrocannabinol (THC) and its active metabolite 11-OH-THC on regional cerebral blood flow in the rat in order to determine functional CNS sites of action for the cannabinoids. Conscious rats were injected IV with one of four doses of THC (0.5, 1, 4, 16 mg/kg), 11-OH-THC (4 mg/kg), or vehicle 30 min prior to sacrifice. Regional cerebral blood flow was determined autoradiographically using the freely diffusible tracer method of Sakaruda et al. Changes in regional cerebral blood flow were observed in 16 of the 37 areas measured. Decreases in regional cerebral blood flow following THC were seen in such areas as the CA1 region of the hippocampus, frontal and medial prefrontal cortex, the nucleus accumbens, and the claustrum. Thresholds for these effects ranged from 0.5 to 16 mg/kg. Areas unaffected by THC include the medial septum, ventral tegmental area, caudate, temporal, parietal and occipital cortex, and cerebellum. These data indicate that THC and its active metabolite, 11-OH-THC, cause a heterogeneous alteration in the activity of specific CNS sites, many of which are involved in the characteristic behavioral actions of THC. © 1997 Elsevier Science Inc.

Δ^9-Tetrahydrocannabinol Cerebral blood flow Rat

MARIJUANA and its principal psychoactive component, Δ^9-tetrahydrocannabinol (THC), have effects on a broad spectrum of physiological systems and body organs, including distinct psychoactive properties. A wide spectrum of characteristic behavioral actions has been described in both humans and other animals [see reviews (9,12)]. In rodents, motor behavior, learning and memory, and body temperature are all affected by THC in a dose range of 0.5–20 mg/kg. In addition, THC enhances electrical brain stimulation reward (16) and has discriminative stimulus properties (4).

Major gains have been made in our understanding of the mechanism of action of the cannabinoids in recent years. A cannabinoid receptor has been identified recently (23), and its distribution in mammalian brain described (18,19). In addition, a cDNA that codes for a cannabinoid receptor has been isolated, its expression studied, and the anatomical localization of the message it produces described (33). An endogenous ligand for the receptor has also been reported (11,15).

Cannabinoid binding sites are found to be widely but heterogeneously distributed throughout the brain, with a distribution unique to any known neurotransmitter (18,19). Highest receptor densities were found in the hippocampus, cerebellum, substantia nigra, and globus pallidus, whereas regions lowest in binding include the hypothalamus and lower brainstem. The distribution of messenger RNA coding for a seemingly identical receptor has also been reported (33), and generally follows the distribution of the cannabinoid receptor; however, moderate to high levels of message are also found in the hypothalamus.

In general, the neuroanatomical localization of cannabinoid receptors correlates with areas that are likely to subserve the pharmacological actions of marijuana. For example, high receptor densities are found in brain areas such as the globus pallidus and cerebellum, which are involved in motor control, and in the hippocampal formation, which has been implicated in memory formation. However, the correlation between the areas in the brain where receptors are located

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and where the cannabinoids act has not been examined fully. Margulies and Hammer (30) reported that THC alters brain metabolism in a biphasic dose-dependent manner. They found that a low dose (0.2 mg/kg) of THC increased 2-deoxyglucose uptake in many cortical and limbic areas, including the hippocampal formation and medial septum. Higher doses (2 and 10 mg/kg) caused a decrease in glucose uptake in most of the brain areas measured, including many neocortical areas, hippocampal formation, amygdala and hypothalamus. In general, the 2 mg/kg dose was more effective than the 10 mg/kg dose. The same authors reported that the potent synthetic cannabinoid, CP-55940, had a similar biphasic effect on cerebral glucose uptake (31).

It is generally agreed that there is a close relationship between brain electrochemical activity, energy metabolism, and brain blood flow, with the relationship between energy metabolism and brain blood flow reflecting the tight coupling between removal of metabolic byproducts and supply and utilization of energy substrates (41). Under normal physiological conditions, the level of blood flow reflects the level of glucose and oxygen consumption. The brain stores virtually none of its energy substrates and is therefore dependent upon continuous blood supply for normal functioning. An autoradiographic method to measure regional cerebral blood flow (rCBF) has been developed using [^{14}C]iodoantipyrine, a freely diffusible tracer that requires only a 30–60-s measurement period (39). We have previously reported that a high dose of THC (16 mg/kg) decreased regional cerebral blood flow in many areas of the brain (5). In order to better determine functional sites of action for the cannabinoids, we now report the dose-dependent effects of THC and its active metabolite, 11-OH-Δ^9-THC on rCBF in the rat. THC doses in a range (0.5–16 mg/kg) that encompasses the major behavioral and physiological of the drug in rodents were used.

METHOD

Thirty male, Sprague–Dawley derived rats (Sasco, Madison, WI) weighing 275–350 g were housed in plastic tubs in a temperature controlled room with lights off between 0830–2030. Food and water were available continuously. Prior to experimentation, each rat experienced a restraint procedure of increasing duration for 5 days, progressing from 1–5 h/day. The restraint consisted of gently immobilizing both fore and hind limbs by wrapping the rat in a terry cloth jacket. Rats acclimated rapidly to this restraint and would accept food and water if offered.

On the day of rCBF determination, rats were anesthetized with Chlorapent® (2.5 ml/kg) (chloralose and pentobarbital) and femoral arterial and venous catheters were implanted in the left leg. Immediately following surgery, animals were placed in restraint and allowed to recover from anesthesia for a minimum of 5 h. An intravenous injection of 500 IU/kg of heparin was delivered in 0.5 ml of saline, 1 h prior to drug treatment.

Groups of rats (n = 4–6 per group) received one of six drug treatments: Δ^9-THC at a dose of 1) 0.5 mg/kg; 2) 1.0 mg/kg; 3) 4 mg/kg; or 4) 16 mg/kg; or 5) 4.0 mg/kg 11-OH-Δ^9-THC; or 6) vehicle. All injections were made by hand into the femoral vein in a volume of 1 ml/kg over a period of approximately 15 s and followed by 0.1 ml of saline to flush the catheter. Cannabinoids, which are virtually insoluble in aqueous media, were prepared using an Emulphor®-ethanol (1:1)-saline vehicle (10). rCBF measurement commenced 30 min after drug or vehicle treatment.

rCBF was measured using the method of Sakurada et al. (39) and described in more detail elsewhere (43). Briefly, this autoradiographic method involved the infusion of a 0.5 ml saline solution containing 100 μCi/kg of [^{14}C]iodoantipyrine (IAP) (45.5 mCi/mmol, Amersham) at a constant rate over 30 s into the femoral vein. Arterial blood samples (approximately 40–60 μl) were collected every 5 s onto preweighed filter paper during the entire 30-s infusion period. Blood flowed freely from the 6—7-mm length of PE 10 tubing, which had a dead space of approximately 3—4 μl. At the end of the IAP infusion period, rats were sacrificed by rapid decapitation and the entire skull, less jaw and fur, frozen in isopentane (–50°C) and stored at −80°C until sectioned. This rapid freezing procedure minimized the diffusion of the IAP, producing excellent spatial resolution. Blood-soaked filter papers were immediately sealed in 7-ml counting vials and reweighed. Radioactivity was determined by liquid scintillation spectroscopy, 24 h after the addition of 5 ml of a toluene based scintillation fluid (Budget-Solve; RPI, Mt. Prospect, IL).

Brains were removed from skulls in a cryostat (Reichert-Jung 1800) and subsequently sectioned at −20°C into 20-μm slices in the coronal plane. Sections were thaw mounted onto glass slides, dried on a slide warmer, and apposed to X-ray film (Kodak MR-1) in standard cassettes with calibrated [^{14}C] methyl methacrylate standards (Amersham) for up to two months. Following film development, slides were stained with thionin for subsequent anatomic localization of regions of interest (ROI). Brain regions were analyzed using a computerized image analyzer (MCID, Imaging Research, St. Catherines, Ontario, Canada) with ROI defined using comparable stained sections as defined by the atlas of Paxinos and Watson (37). Five evenly spaced bilateral densitometric measurements were taken for each region analyzed and averaged together for each rat.

Regional cerebral blood flow (ml/100 g/min) was calculated on-line with the aid of the blood flow radioactivity and optical density calibration curves using the operational equation of Sakurada (39). No correction for catheter dead space was made because Jay et al. (24) demonstrated that catheter flow rates at least 40 times the dead space volume accurately represented brain blood flow. Our ratios generally ranged from 60 to 90 times dead space.

Heart rate and mean arterial, diastolic, and systolic blood pressure were determined during the experimental period using a Statham pressure transducer and Grass Model 79D polygraph connected to the femoral arterial catheter. Arterial blood samples were also collected prior to drug administration and at the time of sacrifice and analyzed for pH, $PaCO_2$, PaO_2 and HCO_{3-} using a model 168 Corning-Ciba Blood Gas Analyzer.

Differences among rCBF treatment groups were evaluated by one-way analysis of variance (ANOVA) over the six drug treatments for each structure analyzed. Tests for simple effects were performed using Fisher's Least Significant Difference (LSD) Test where appropriate. A significance level of $p \leqslant 0.05$ was used in all studies. Alterations in physiological parameters were assessed using a repeated measures ANOVA.

RESULTS

The effects of all but the lowest dose Δ^9-THC and of 11-OH-Δ^9-THC were apparent upon gross behavioral observation. Rats demonstrated the commonly reported increased response to environmental stimuli as well as an increase in spontaneous vocalizations. Separate univariate ANOVAs indicated that Δ^9-THC and/or 11-OH-Δ^9-THC altered rCBF in 16 of the 37 brain areas measured, with a threshold to effect that varied between 0.5 and 16 mg/kg depending on the brain area. The results of these studies are shown in Table 1.

Most affected areas demonstrated a decrease in rCBF in response to THC. The greatest changes were seen in the hippocampus, claustrum, and medial prefrontal cortex, where rCBF was decreased by 35–39% at the 16 mg/kg dose. Effects of a similar magnitude (38–43% decrease) were caused by 11-OH-Δ^9-THC in these same areas. Significant decreases in rCBF were observed after the 0.5 mg/dose in the claustrum and after 1 mg/kg in the rostral medial prefrontal cortex, rostral nucleus accumbens, and frontal cortex. A 4 mg/kg threshold was seen in the CA1 region of the hippocampus and the caudal nucleus accumbens. rCBF also decreased in the dentate, entorhinal cortex, and the globus pallidus, but only after the highest (16 mg/kg) dose used. The basolateral nucleus of the amygdala and the agranular insular cortex were affected only by 11-OH-Δ^9-THC.

In contrast, a statistically significant increase in rCBF, when compared with the vehicle-treated group, was seen with the 4 mg/kg dose in the arcuate nucleus (also with the 0.5 mg/kg dose in white matter). The biological significance of these single dose effects is not clear. Other hypothalamic areas were

TABLE 1

REGIONAL CEREBRAL BLOOD FLOW

	Δ^9-THC					11-OH-THC
Brain region	Vehicle (n = 6)	0.5 mg/kg (n = 6)	1.0 mg/kg (n = 5)	4.0 mg/kg (n = 4)	16.0 mg/kg (n = 4)	4.0 mg/kg (n = 5)
Mesocorticolimbic						
Amygdala–basolateral nucleus	142 ± 10	143 ± 7	119 ± 7	133 ± 13	118 ± 8	109 ± 6*
Bed nucleus of stria terminalis	88 ± 5	97 ± 4	90 ± 6	95 ± 11	84 ± 10	83 ± 4
Dentate gyrus	111 ± 8	120 ± 9	97 ± 8	107 ± 10	82 ± 3*	91 ± 4
Diagonal band of broca	169 ± 16	192 ± 13	188 ± 10	171 ± 14	170 ± 14	161 ± 11
Hippocampus–CA1	112 ± 8	113 ± 5	97 ± 8	85 ± 18*	72 ± 7*	85 ± 3*
Hippocampus–CA3	109 ± 12	126 ± 4	107 ± 7	92 ± 21	84 ± 8	97 ± 2
Lateral septum	135 ± 18	143 ± 9	126 ± 10	121 ± 13	119 ± 12	110 ± 6
Medial septum	173 ± 14	194 ± 14	177 ± 11	162 ± 15	159 ± 19	150 ± 11
Nucleus accumbens (rostral)	222 ± 23	185 ± 10	165 ± 14*	162 ± 19*	140 ± 19*	142 ± 12*
Nucleus accumbens (caudal)	180 ± 16	175 ± 11	155 ± 11	140 ± 11*	127 ± 8*	139 ± 10*
Olfactory tubercle	164 ± 16	162 ± 10	150 ± 11	132 ± 11	117 ± 4	114 ± 10
Ventral tegmental area	145 ± 7	146 ± 6	139 ± 8	154 ± 15	127 ± 6	140 ± 6
Neocortical						
Agranular insular cortex	208 ± 23	221 ± 19	192 ± 19	159 ± 27	177 ± 17	129 ± 3*
Claustrum	284 ± 38	199 ± 15*	196 ± 17*	175 ± 29*	174 ± 14*	162 ± 16*
Entorhinal cortex	149 ± 16	134 ± 6	125 ± 4	128 ± 13	110 ± 16*	103 ± 2*
Frontal cortex (rostral)	282 ± 29	227 ± 25	184 ± 11*	211 ± 31*	216 ± 21	116 ± 14*
Frontal cortex (caudal)	268 ± 37	237 ± 22	185 ± 20*	192 ± 25*	203 ± 19	159 ± 9*
Medial prefrontal cortex (rostral)	283 ± 30	239 ± 18	202 ± 16*	185 ± 20*	186 ± 19*	171 ± 9*
Medial prefrontal cortex (caudal)	237 ± 24	234 ± 18	197 ± 21	183 ± 19	180 ± 16	158 ± 10*
Occipital cortex	259 ± 31	237 ± 22	202 ± 16	186 ± 39	213 ± 23	166 ± 9
Parietal cortex	238 ± 33	242 ± 21	196 ± 17	208 ± 36	201 ± 13	176 ± 4
Temporal cortex	283 ± 34	295 ± 20	242 ± 19	258 ± 41	254 ± 21	211 ± 11
Hypothalamus						
Anterior nucleus	137 ± 18	146 ± 7	134 ± 9	122 ± 15	103 ± 13	123 ± 7
Arcuate nucleus	115 ± 14	146 ± 14	118 ± 5	153 ± 20*	97 ± 10	116 ± 3
Dorsal medial nucleus	131 ± 20	146 ± 9	127 ± 12	117 ± 13	82 ± 8	121 ± 5
Lateral nucleus	130 ± 11	144 ± 5	131 ± 11	122 ± 17	109 ± 7	116 ± 5
Medial preoptic area	105 ± 11	114 ± 6	100 ± 7	106 ± 15	93 ± 16	90 ± 5
Posterior nucleus	139 ± 6	153 ± 9	124 ± 9	158 ± 10	117 ± 4	120 ± 4
Ventral medial nucleus	123 ± 20	139 ± 7	144 ± 17	118 ± 13	90 ± 6	131 ± 5
Nigrostriatal						
Caudate nucleus	180 ± 14	160 ± 8	149 ± 12	154 ± 11	134 ± 9	138 ± 8
Globus pallidus	87 ± 5	98 ± 5	81 ± 5	90 ± 9	71 ± 4*	82 ± 3
Substantia nigra pars reticulata	122 ± 8	123 ± 7	111 ± 8	122 ± 13	92 ± 5	109 ± 5
Substantia nigra pars compacta	136 ± 8	137 ± 6	128 ± 9	139 ± 11	113 ± 6	133 ± 5
Cerebellum						
Medial molecular	141 ± 12	137 ± 5	138 ± 19	N.D.	N.D.	107 ± 8
Medial granular	171 ± 12	163 ± 10	155 ± 13	N.D.	N.D.	156 ± 14
Lateral molecular	128 ± 9	111 ± 5	112 ± 9	N.D.	N.D.	102 ± 7
White matter	55 ± 4	68 ± 5*	57 ± 2	55 ± 6	42 ± 2	51 ± 2

Data represent the mean ± SEM of rCBF in ml/100g/min.
N.D. - not determined.
*$p \leq .05$ when compared to vehicle treated group.

not affected. THC administration did not have a significant effect on any of the cerebellar or nigrostriatal (other than the globus pallidus) areas analyzed. Other areas such as olfactory tubercle, ventral tegmental area, and septal area also showed no significant changes after drug administration.

The effects of cannabinoids on physiological parameters are shown in Table 2. Although mean arterial blood pressure was not altered, significant decreases in heart rate were observed at 30 min after cannabinoid treatment. The greatest decrease was produced by the 16 mg/kg dose of THC. Arterial pCO_2 was significantly increased by all doses of THC and by 11-OH-Δ^9-THC. The largest increase was observed after treatment with 11-OH-Δ^9-THC. Blood pH was decreased only by 11-OH-Δ^9-THC. No other significant alterations were observed.

DISCUSSION

The results of this study suggest that neuronal activity, as measured by changes in rCBF, is altered by Δ^9-THC and its active metabolite, 11-OH-Δ^9-THC in a regionally specific manner. These data, in contrast to receptor binding and mRNA hybridization experiments, provide a functional map of cannabinoid action. It is noteworthy that many of the brain regions displaying altered rCBF have also been implicated in the major behavioral and physiological effects produced by the cannabinoids, such as enhancement of electrical brain stimulation, impairment of short-term memory, and altered endocrine function.

Good agreement exists between those brain areas having high densities of cannabinoid receptors (18,19) and brain areas that are thought to subserve many of the behavioral and physiological effects of Δ^9-THC. Receptors in the basal ganglia and cerebellum may be involved in cannabinoid motor effects, such as static ataxia in dogs (12) and decreased spontaneous activity and catalepsy in mice (28). Decreased rCBF observed in the globus pallidus in the present study might reflect this altered motor response following THC treatment. The reasons for the lack of statistically significant effects in the cerebellum are not clear. However, the effects of the highest doses of Δ^9-THC on this region were not determined. Volkow (47) reported that THC increased metabolism in the cerebellum in humans using positron emission tomography (PET).

In general, the brain areas that we found to be affected by THC in this study are explicable based upon our knowledge of the localization of cannabinoid receptors and the physiological and behavioral effects of this class of drugs, although there were some obvious exceptions. For example, rCBF was decreased significantly in the CA1 area of the hippocampal formation, an area dense in cannabinoid receptors (19). However, rCBF was not affected significantly in the hippocampal CA3 area and was decreased only by the 16 mg/kg dose in the dentate gyrus. Although all three regions have a high density of cannabinoid receptors, each receive different neuronal inputs. There are three major excitatory pathways ultimately connecting the subiculum to the CA1 region of the hippocampus (26). There are also inputs from many other areas, such as the septum, where rCBF was not affected in the present study. Thus, the decrease in rCBF in the CA1 region may be due to the summation of decreased activity in other afferent areas that ultimately terminate in the CA1 region. It has been suggested that a large part of the changes measured using metabolic and rCBF autoradiographic techniques appear to be due to presynaptic activity within a region and, therefore, observed alterations in metabolic activity are likely due mostly to changes in afferent inputs to that structure and less so to intrinsic or efferent projection neurons contained within the structure (25,32).

There are many studies suggesting that rCBF in the hippocampus and other related cortical structures might be affected by Δ^9-THC. For example, several investigators have reported that hippocampal electrophysiology is affected both in vivo and in vitro by Δ^9-THC (6,14,36,46). In these studies, depressed evoked responses were seen following high THC doses or concentrations, whereas increases were reported at lower drug doses. In vivo doses to rodents in these studies were in the 1.25–16 mg/kg range (38), similar to those used in the current study. In the present study, rCBF in the hippocampal CA1 region was decreased significantly, as much as 36% by THC doses of 4 mg/kg and greater. In contrast to the

TABLE 2

CANNABINOID EFFECTS ON PHYSIOLOGICAL PARAMETERS

		Δ^9-THC					11-OH-THC
Variable	Time (min)	Vehicle	0.5 mg/kg	1.0 mg/kg	4.0 mg/kg	16 mg/kg	4 mg/kg
Heart rate (beats/min)	0	447 ± 19	445 ± 10	438 ± 6	352 ± 7	413 ± 31	468 ± 71
	30	469 ± 20	382 ± 12*	377 ± 14*	320 ± 37	275 ± 39*	376 ± 66*
Mean blood pressure	0	127 ± 5	130 ± 7	121 ± 8	121 ± 5	123 ± 4	133 ± 9
	30	126 ± 5	124 ± 6	119 ± 12	122 ± 10	114 ± 9	141 ± 18
Pa_{O2}	0	88.6 ± 2.8	82.6 ± 2.1	92.1 ± 7.9	102.4 ± 5.9	90.5 ± 6.1	86.2 ± 2.7
	30	88.6 ± 3.0	87.0 ± 3.5	92.1 ± 5.0	101.2 ± 6.9	87.7 ± 7.3	82.5 ± 3.0
Pa_{CO2}	0	34.4 ± 1.9	37.9 ± 1.0	33.8 ± 2.6	35.0 + 0.7	36.7 ± 1.2	38.1 ± 2.4
	30	36.0 ± 1.1	42.6 ± 1.4*	40.3 ± 3.0*	39.5 ± 1.4*	42.2 ± 3.1*	47.3 ± 1.4*
HCO_3-	0	21.4 ± 1.4	24.9 ± 0.9	22.1 ± 1.9	20.4 ± 1.5	20.4 ± 1.3	23.3 ± 0.6
	30	21.6 ± 1.4	25.9 ± 0.6	24.8 ± 1.1	20.1 ± 1.6	22.9 ± 0.6	24.8 ± 0.5
pH	0	7.40 ± .01	7.43 ± .01	7.42 ± .03	7.46 ± .03	7.36 ± .02	7.40 ± .02
	30	7.39 ± .03	7.39 ± .02	7.41 ± .02	7.47 ± .03	7.35 ± .03	7.33 ± .02*

Effects of Δ^9-THC and 11-OH-Δ^9-THC on cardiovascular and arterial blood gas variables. The values shown are the mean ± the S.E.M. with an n of 4 to 6 at each dose. Significant main effects were determined using analysis of variance for repeated measures. Individual comparisons were made using Fisher's LSD test.

*$p < .05$ when compared to 0 time for that dose.

LCGU study of Margulies and Hammer (30), we did not observe significant increases in rCBF in the hippocampus. However, this may be due to the fact that we did not examine the effects of doses less than 0.5 mg/kg, the range in which they reported stimulatory effects.

The hippocampus has been implicated as being involved prominently in the storage of memories across most mammalian species (42,44). Δ^9-THC has been shown to impair short-term memory in rodents, monkeys, and humans (3,35). Memory consolidation may also be affected by THC (3). Thus, the decrease in hippocampal rCBF after THC treatment is understandable in terms of the impairment of hippocampal function produced by THC.

Marijuana produces a characteristic euphoria or "marijuana high" in man (21) and enhances brain reward mechanisms in rats (16). Significant decreases in rCBF were observed in cortical areas such as the frontal and medial prefrontal cortex. rCBF was also decreased in the nucleus accumbens, a mesolimbic field that receives significant input from among other regions, the medial prefrontal cortex. The medial prefrontal cortex receives major inputs from the ventral tegmental area, amygdala, claustrum, lateral hypothalamus, and the CA1 region of the hippocampus. In the current study, rCBF was decreased in several of these structures, including the claustrum, amygdala, and CA1 region, which may have contributed to the rCBF response in the medial prefrontal cortex. Many of these areas are involved in the ventral tegmental area–nucleus accumbens–ventral pallidum reward network. The interactions of cannabinoids with this system have been reviewed by Gardner (16). It was reported that THC, like many other abuse prone drugs, enhances electrical stimulation brain reward in Lewis rats (17). These investigators (7,8) also reported that THC enhances dopamine release from reward relevant areas, including nucleus accumbens and medial prefrontal cortex. These effects were seen at doses of 0.5 to 2 mg/kg, with peak effects occurring prior to 60 min.

The prefrontal cortex appears to be involved in the execution of several behaviors, including programming new behaviors, suppressing interfering stimuli, and organizing temporal and spatial concepts of information (42). Lesions in this area can produce an impairment in a variety of delay type tests. THC appears able to impair these types of behaviors in a variety of species, including humans. In monkeys, THC impaired performance on a temporal response differentiation task relative to controls (40). The ability of cannabis to impair time perception (45) and vigilance or sustained attention over time (1) is well demonstrated. Memory intrusions can be caused by Δ^9-THC, indicating that the memory process is more susceptible to interference (22). Thus, THC not only impairs behaviors associated with medial prefrontal cortex, it also decreased rCBF significantly both in this region, as well as in a number of prominent afferents into the medial prefrontal cortex.

The relatively low density of cannabinoid receptors in the hypothalamus (18), coupled with the general lack of effect of THC on rCBF in hypothalamic areas in the present study, is of particular interest. These data are curious in light of the well-documented endocrine actions of Δ^9-THC (12,21,34). Margulies and Hammer (30) also reported alterations in LCGU after only a single intermediate dose of THC. There are several possible reasons for the lack of observable THC effects in this study. For example, the endocrine hypothalamus is known as a high gain system that requires only relatively modest changes in neuronal firing to induce releasing factors, which then go on to produce large changes in systemic hormone levels. It is likely that these small changes in the number of action potentials would be lost among the "metabolic noise" of this heterogeneous region of the brain.

Our measurements of blood gases and cardiovascular responses indicate that changes in rCBF observed after THC administration were likely not secondary to changes in cardiovascular function. A decrease in heart rate, typical of Δ^9-THC administration to rodents (2), was seen after all cannabinoid treatments. However, because mean arterial blood pressure did not differ significantly from predrug levels at the time of rCBF measurement, it is unlikely that changes in brain perfusion occurred as a result of drug-induced bradycardia. Significant increases in arterial pCO_2 were also observed after treatment with all cannabinoid doses. As a vasodilator, these increases would be expected to result in homogeneous increases in cerebral blood flow of about 3–4% per mm Hg change in arterial PCO_2 (27), rather than the selective decreases in rCBF that we observed. If anything, increases in arterial pCO_2 may have led to an underestimate of the number of drug-induced changes in rCBF rather than produce false positives. Thus, the direction and heterogeneity of the changes in rCBF that we observed suggest that they are the result of drug effects in the brain, rather than a response to changes in peripheral physiology.

The results of the current study are in general agreement with those reported previously, which examined the effects of Δ^9-THC on local cerebral glucose utilization. Margulies and Hammer (30) observed decreased glucose utilization in many brain areas, including the hippocampus and neocortex, following moderate to high doses of THC (2 and 10 mg/kg). All structures common to both reports demonstrated decreased metabolic activity. However, regions of decreased glucose utilization reported by Margulies and Hammer were more widespread than in the present study including areas of the neocortex and hypothalamus. Few significant decreases (3 of 42 areas) were seen at their 0.5 mg/kg dose, as was the case in the present study (1 of 37 areas).

Margulies and Hammer also reported that, at the lowest dose of Δ^9-THC used (0.2 mg/kg), increases rather than decreases in glucose utilization were seen in several brain areas including the hippocampus, neocortex, and limbic areas, but not in most hypothalamic areas. In the present study, statistically significant increases in rCBF were found only in the arcuate nucleus (at 4 mg/kg) and white matter (at 0.5 mg/kg). However, it is of interest that observable (greater than 10% or 10 ml/100 g/min), but not statistically significant, increases in rCBF were seen in 15 of 37 structures measured at the 0.5 mg/kg dose; lower doses were not examined. The reasons for the differences in results between the two studies are not clear, although they may be due to methodological differences.

Herkenham et al. (19) described a distribution of cannabinoid receptors that included most of the structures found to be affected in the present study. They also found significant densities of receptors in structures where rCBF was not altered. The misalignment of receptor localization and drug effects on metabolism is not unusual or totally unexpected. As stated above, nerve terminals are thought to represent a major site of metabolic activity. Thus, metabolic and blood flow changes in a particular brain region are likely to be due to alterations in afferent input to that area rather than direct drug interactions with receptors on perikarya in that structure. Thus, there is no necessary relationship between the localization of drug receptors in a region and changes in metabolism and rCBF caused by that drug.

Finally, it is thought that not all of the pharmacological and neurochemical actions of Δ^9-THC and other cannabinoids

are mediated by the cannabinoid receptor (13,29). The structure–activity relationships for some pharmacological and chemical effects support this concept (see reviews (12,38)). The nonreceptor-mediated actions may be due to an effect of these highly lipophilic compounds on membranes (20) or another as yet unknown mechanism. Thus, it is possible that some of the alterations in rCBF observed in the present study may have been due, at least in part, to cannabinoid actions that are not mediated by the cannabinoid receptor. This is most likely for changes seen at the higher doses (4 and 16 mg/kg) studied.

In summary, the results of this study indicate that Δ^9-THC and its active metabolite, 11-OH-Δ^9-THC, produce regionally specific alterations in the functional activity of the rat brain. These effects are localized in brain areas that may be involved in many of the behavioral and physiological actions of the cannabinoids. However, because metabolic and rCBF mapping techniques cannot identify primary sites of drug action unambiguously, further studies are needed to determine these sites and the primary neurochemical mechanisms involved in the mediation of the actions of both low and high doses of the cannabinoids.

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