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THE UPTAKE OF Δ^9-TETRAHYDROCANNABINOL IN CHOROID PLEXUS AND BRAIN CORTEX *IN VITRO* AND *IN VIVO*

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SUMMARY

[^{3}H]Δ^9 Tetrahydrocannabinol (Δ^9-THC) was actively transported by the choroid plexus and cerebral cortical slices of the rabbit when incubated as a BSA-microsuspension in artificial rabbit CSF. The transport system for Δ^9-THC in choroid plexus had a V_{max} of 174 nmoles/mg tissue/h, approximately 9-fold greater than that observed for cortical slices. *In vivo* experiments demonstrated a preferential distribution of Δ^9-THC in choroid plexus at 1 h after intravenous injection. These results indicate that Δ^9-THC is actively accumulated by choroidal epithelium and may also be transported across the epithelial stroma into the capillary circulation. This suggests that the choroid plexus participates in the regulation of Δ^9-THC concentration in CSF and indirectly in brain by means of the 'sink' function of the CSF.

INTRODUCTION

Among the wide variety of substances reported to be actively transported by the choroid plexus are a number of narcotic and hallucinogenic drugs, including morphine[3,9,15,27,29], dihydromorphine[15,25], codeine[15], lysergic acid diethylamide (LSD)[11,30], and methadone[14]. Regional variations in brain distributions of Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive component of marijuana, have been reported following its parenteral administration in animals[19]. Although labeled Δ^9-THC has been detected in the choroid plexus by autoradiography[19], studies on its specific uptake compared to other parts of the brain have not been reported. In view of the known transport of the above mentioned drugs by the choroid plexus and also because of the clinical report of cerebral ventricular dilatation in chronic marijuana users[4], we felt it of interest to investigate the possibility of active

transport and the nature of metabolism of Δ^9-THC by choroid plexus. The present report records studies on the uptake and metabolism of [^{3}H]Δ^9-THC *in vitro* and *in vivo* by the choroid plexus as compared to cerebral cortex of the rabbit.

METHODS

The water insolubility of Δ^9-THC necessitates the addition of vehicles such as Tween 80, alcohol, ethylene glycol and bovine serum albumin (BSA) for its intravenous administration. This problem is compounded when Δ^9-THC is studied in isolated tissue preparations by the problem of attaining adequate suspensions of this compound in standard incubation media. Various vehicles (alcohol, Tween 80 and BSA) and methods of filtration were tested in preliminary experiments and the following procedure proved to be the most satisfactory. A 100% alcohol solution of [^{3}H]Δ^9-THC (0.1 μCi/μg)* was used to prepare a microsuspension of the Δ^9-THC by a modification of the method of Perez-Reyes *et al.*[22]. A stock 1 mg/ml solution was made by dissolving 4 mg of the original [^{3}H]Δ^9-THC in 4 ml 100% ethanol. For incubations, 150-μl aliquots of the stock isotope solution were evaporated to dryness under nitrogen and the residue suspended in 0.3 ml of 10% BSA. The suspension was mixed with 30 ml of artificial rabbit CSF[23], with vigorous stirring on a vortex stirrer, followed by 10 min sonication and filtration through a 0.45-μm Millipore filter. The final suspension contained approximately 0.26 μCi/ml [^{3}H]Δ^9-THC with a BSA concentration of 100 mg%. For saturation kinetic studies, the medium concentration was increased by the addition of ethyl alcohol to the BSA suspension to give a solution of 1% alcohol to which was added appropriate amounts of unlabeled Δ^9-THC and filtered with a 3.0-μm Millipore filter. This procedure yielded a filtrate concentration of Δ^9-THC of 185 μM as calculated from specific activity as compared to 28 μM using the 0.45 μm filter.

In vitro experiments

Male, New Zealand white rabbits weighing between 1.5 and 2.2 kg were killed by the insufflation of 30 ml of air into an aural vein followed by the severance of the cervical vessels. The calvarium was rapidly removed, the lateral ventricles opened by bilateral incisions and the choroid plexus gently excised. Samples of superficial parietal cortex were also collected from each hemisphere. Tissues were rinsed with artificial CSF prior to incubation.

Incubations. Tissue incubations were carried out as previously described[2] using artificial rabbit CSF which had been equilibrated with 5% CO_2, 95% O_2 to pH of 7.4 just before use. Brain cortex was cut into 250-μm sections with a Sorvall Tissue Chopper prior to incubation, whereas the choroid plexuses were incubated intact. Incubation vials contained 0.26 μCi/ml in 2 ml of artificial CSF. Most incubations were carried out at 37 °C for 1 h in an atmosphere of 5% CO_2, 95% O_2 with gentle

* Both labeled and unlabeled Δ^9-THC were obtained through the courtesy of Dr. Colin Pitt, Chemistry and Life Science Laboratory, Research Triangle Institute, N.C., U.S.A.

shaking in a Dubnoff metabolic incubator. Other experiments included time courses and reversibility studies. After incubation the tissues were rinsed 3 times in non-radioactive artificial CSF, blotted on filter paper and weighed in tared vials. Liquid scintillation counting was carried out after digestion of the tissues in 0.5 ml Protosol (New England Nuclear Corp.) at 55 °C for 16 h. The digesta were neutralized with glacial acetic acid followed by the addition of 15 ml of Aquasol (New England Nuclear Corp.) before determination of radioactivity. Counts were made to less than 1% error using a Packard Model 3310 Liquid Scintillation Spectrometer. Samples of incubation media were treated identically and counted in triplicate. Correction for quenching was made by an external standard attachment on the instrument. The uptake of [^{3}H]Δ^9-THC was expressed as tissue–media (T/M) ratios:

$$T/M = \frac{\text{counts/min/mg tissue}}{\text{counts/min/}\mu\text{l media}}.$$

Metabolic inhibitors. Solutions of metabolic inhibitors were prepared with artificial CSF as solvent and added to media flasks in concentration as indicated in Fig. 2. Inhibitors used were ouabain, *p*-chloromercuribenzoate (PCMB) (Calbiochem Co.) and KCN and iodoacetic acid (Eastman Kodak). In inhibition experiments the plexus from one lateral ventricle was incubated with medium containing an inhibitor and that from the other ventricle was used as a paired control in media without inhibitor. Brain slices were similarly paired.

Binding studies. Application of conventional methods of assessing the extent of protein-bound radioactivity in incubated tissues was complicated in this study by the fact that a BSA-microsuspension was employed as the Δ^9-THC vehicle. In preliminary experiments, the radioactivity of preincubation BSA-microsuspension of [^{3}H]Δ^9-THC was 73 and 75.5% protein bound as determined by dialysis and TCA precipitation respectively*. Investigation on the extent and nature of postincubation binding were carried out by means of centrifugation, Bio-gel P-2 column chromatography and reversibility studies. Protein determinations were carried out by the method of Lowry *et al.*[18].

Thin layer chromatography experiments on methanol extractions of stock solutions of Δ^9-THC and incubated tissues were made by the method of Ho *et al.*[12], modified by the use of 1:1 chloroform–acetone solvent system with a silica gel plate.

In vivo experiments

Fifty μl (5 μCi) of Δ^9-THC in a 67% alcohol solution made up with 0.9% NaCl in a volume of 0.5 ml was injected into the ear vein of 5 unanesthetized rabbits. Heparinized blood samples were taken from the opposite ear at 15-min intervals for monitoring of plasma and whole blood levels of radioactivity. At 1 h after the

* In preliminary experiments employing Tween 80 as a vehicle, good suspensions were obtained as evidenced by a single radioactivity peak in P-2 gel column chromatographs. However, choroid plexus incubations using this vehicle yielded a mean T/M of 1.7, much lower than for BSA-microsuspensions (see Results). Accordingly the latter vehicle was used in these experiments.

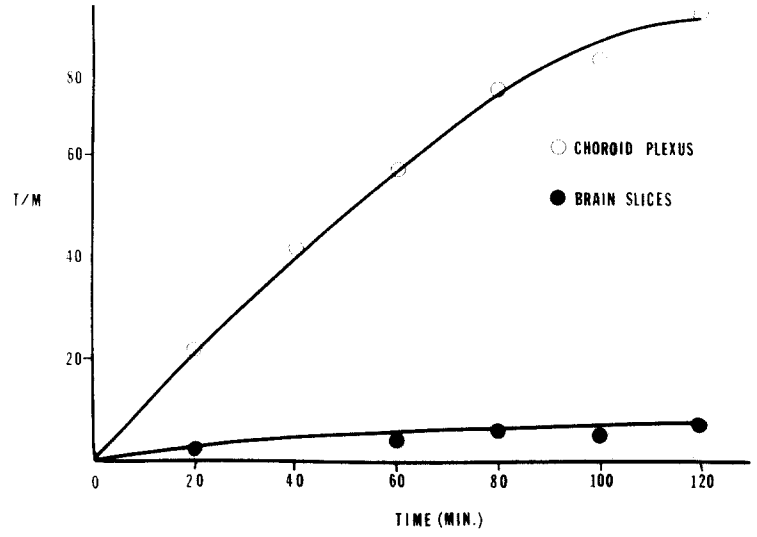

Fig. 1. Changes in tissue–medium ratio (T/M) with different times of incubation. Open circles, choroid plexus; closed circles, brain cortex slices. Each point represents means of two tissues from each of 6 animals used in the same experiment.

isotope injection the animals were anesthetized with Nembutal (25 mg/kg) and exsanguinated by severing cervical vessels. Duplicate samples of choroid plexuses of the lateral ventricles, cerebellar cortex, corpus callosum, parietal and frontal cortex, midbrain, liver and kidney were collected. They were placed in tared glass counting vials, weighed and prepared for counting as described for *in vitro* experiments. Results were expressed as tissue–plasma T/P ratios:

$$T/P = \frac{\text{counts/min/mg tissue}}{\text{counts/min/}\mu\text{l plasma}}$$

RESULTS

In vitro experiments

The time course of radioactivity uptake following incubation of rabbit lateral ventricle choroid plexus and brain cortex slices with [^{3}H]Δ^9-THC is shown in Fig. 1. The T/M values for both tissues increased in an almost linear fashion to 2-h peaks of 88.2 and 7.6 for plexus and brain, respectively. Most subsequent experiments, including transport kinetic studies, were conducted with 60-min incubations.

Fig. 2 demonstrates the accumulation of label against a concentration gradient by both choroid plexus and brain during the 60-min incubations. Choroid plexus T/M values were significantly reduced from control values by all 4 metabolic inhibitors tested and also by incubation at 4 °C. Brain cortex T/M values were also significantly inhibited, although to a lesser extent than choroid plexus, by PCMB (0.15 m*M*), KCN (2 m*M*), iodoacetate (20 m*M*), and 4 °C incubation but curiously not by oua-

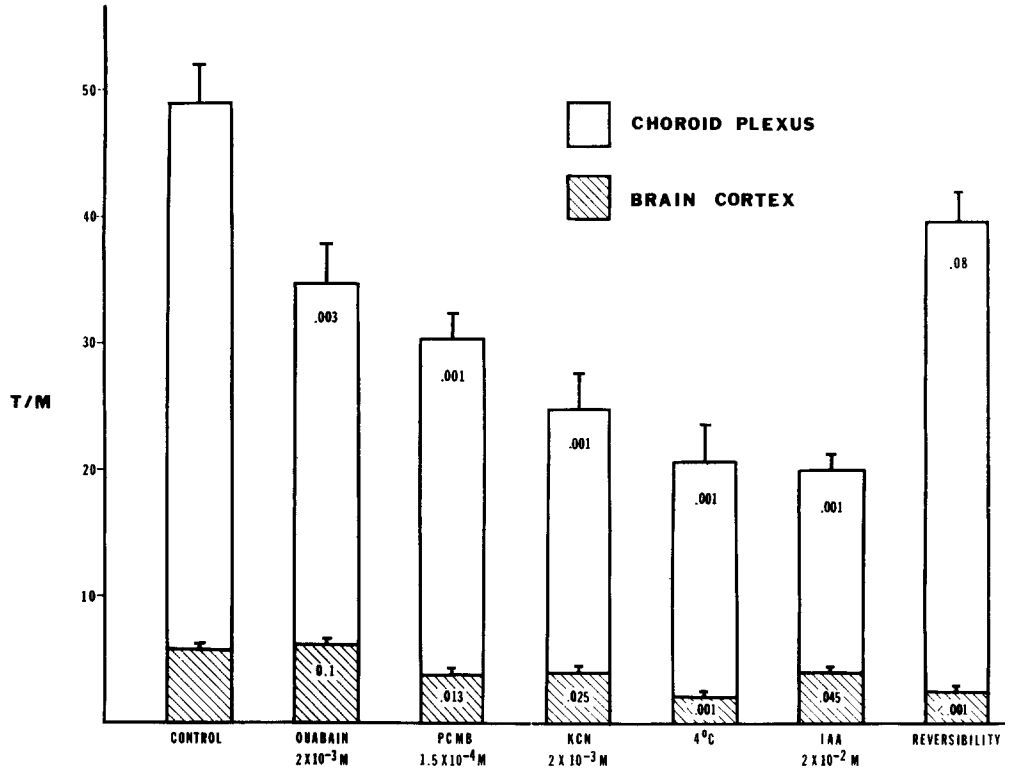

Fig. 2. The effect of various agents on the mean [^{3}H]Δ^9-THC T/M ratios of choroid plexus (open columns) and brain cortex (cross-hatched columns). Bars indicate S.E.; N = 5–12. In reversibility experiments the tissues were incubated with media containing [^{3}H]Δ^9-THC for 1 h, rinsed and incubated in media containing 170 μM non-radioactive Δ^9-THC for an additional 1 h.

bain (2 m*M*). Reversibility of radioactivity uptake was assessed by incubation of the tissues for 1 h in the presence of ^{3}H-labeled Δ^9-THC, rinsing 3 times followed by a second 1-h incubation in media containing 170 μM of unlabeled Δ^9-THC. As indicated in the last column of Fig. 2 the activity of choroid plexus so treated was reduced slightly but not significantly from the control T/M value. On the other hand the T/M of brain cortex slices was significantly reduced following the second incubation. These results indicated partial reversibility of Δ^9-THC activity in brain with slight, but not significant, reversibility of binding in the choroid plexus.

Metabolism of Δ^9-THC in tissues. Thin layer chromatography experiments carried out on choroid plexus and brain cortex following 1-h incubations with [^{3}H]Δ^9-THC gave an R_f value of 0.69 which was identical to that observed in chromatographs of the stock solution of the labeled compound, indicating no metabolism of [^{3}H]Δ^9-THC by either tissue *in vitro*. This is in agreement with previous observations of the absence or negligible metabolism of Δ^9-THC by brain *in vitro*[7,16].

Saturation studies. The kinetics of Δ^9-THC uptake in choroid plexus and brain is indicated in Fig. 3. The uptake of [^{3}H]Δ^9-THC concentration in the incubating

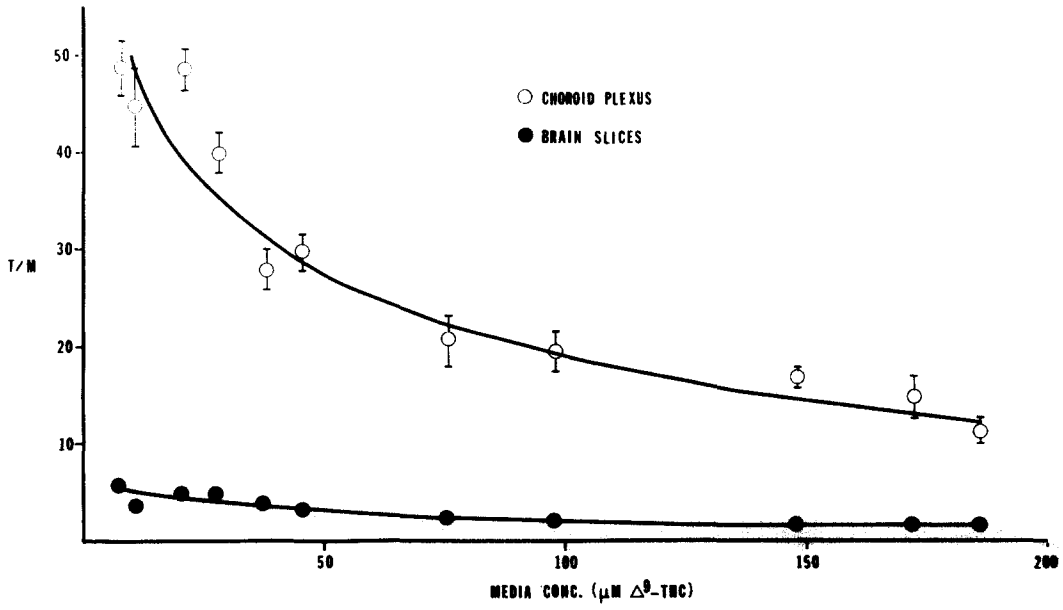

Fig. 3. Reduction of T/M ratios with increasing Δ^9-THC concentrations of incubation media. Open circles, choroid plexus; closed circles, brain cortex. N = 4–8. S.E. is indicated by bars except when the width of the circle exceeded the value.

medium was analyzed by the method of Sampath and Neff[24]. As the concentration in the media was increased from 8 μM to 185 μM the T/M values for choroid plexus declined from a value of 49 to 11, and from 5.5 to 1.5 for brain. Fig. 4A and B present the relationship between total uptake (V_t) of the tissues and media concentration (S) of Δ^9-THC. Both tissues exhibited an exponential (saturable) and a linear (unsaturable) component. Hofstee plots[13] were obtained of the saturable portions of total uptake curves (below 50 μM Δ^9-THC in both cases). At Δ^9-THC concentrations below 50 μM uptake by diffusion was minimal compared to uptake by active transport. Linear regression curves were drawn by the method of least squares, from which apparent transport constants (K_t) of 19.0 and 23.0 μM and V_{max} values of 174 and 20 nmoles/mg tissue/h for choroid plexus and brain respectively were obtained (Fig. 4, insets).

Binding studies. Fig. 5A and B present P-2 gel column chromatographs of the BSA–[^{3}H]Δ^9-THC media before and after 120 min incubation. The bulk of the radioactivity was associated with the protein peak both before and after incubation, indicating that the suspension was apparently unchanged by the 2-h incubation. The secondary radioactive peak represents non-protein-associated activity which appears to agree in magnitude with that in previously mentioned dialysis and TCA precipitation measurements, *i.e.* approximately 75% of the total radioactivity was BSA-associated. Since the uptake of labeled albumin by the isolated plexus is very limited [T/M < 1.0][26] the BSA–Δ^9-THC suspension employed in the present study was apparently sufficiently labile to provide adequate levels of Δ^9-THC in a diffusible state.

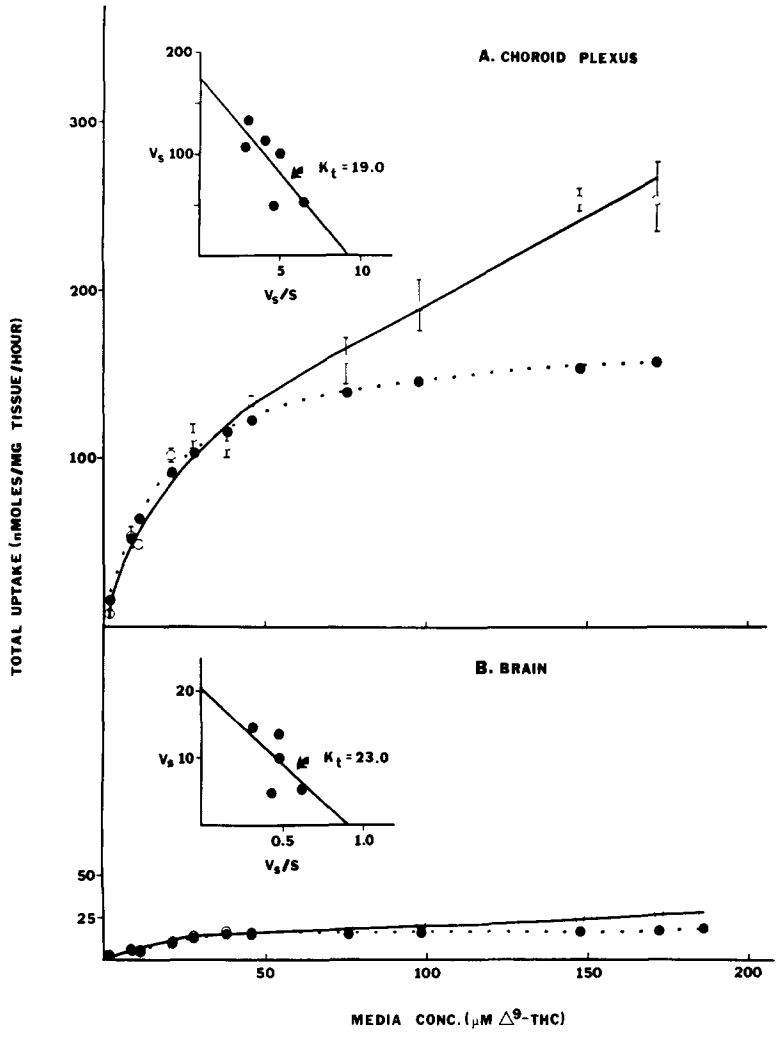

Fig. 4. Relationship between total uptake of Δ^9-THC by, A, choroid plexus and, B, brain slices, and media concentrations of Δ^9-THC. Open circles, experimental values; closed circles, calculated values. Hofstee plots of saturable portion (with media concentrations (S), below 50 μM)) of total uptake (V_s) *versus* V_s/S are presented in the insets of A and B for each tissue. The regression lines were drawn by the method of least squares and the coefficient of correlation values were —0.75 for choroid plexus and —0.56 for brain cortex. Both tissues were incubated 60 min for all determinations. Values represent means ± S.E. except when the width of the circle exceeded the value; N = 4–8.

Fig. 6 demonstrates the time of distributions of [^{3}H]Δ^9-THC following centrifugation of choroid plexus Tris (pH 7.4) homogenates prepared after various incubations. The uptake of Δ^9-THC increased linearly with time of incubation in all fractions with the preponderance of the radioactivity present in the Tris-insoluble fractions.

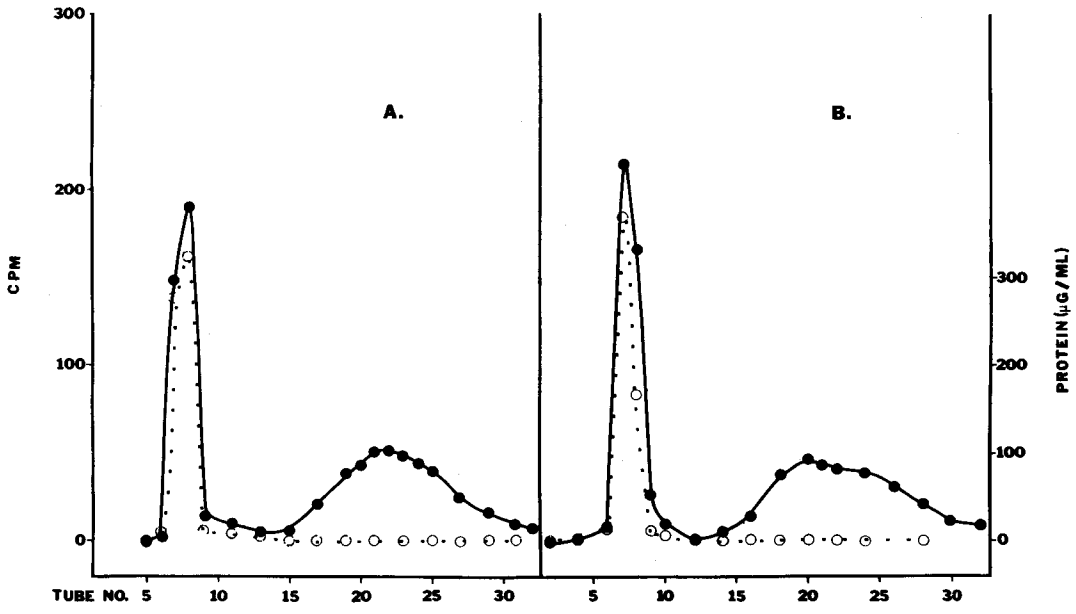

Fig. 5. Bio-gel P-2 column chromatographs of BSA–[^{3}H]Δ^9-THC media before (A) and 120 min after (B) choroid plexus incubation. Closed circles, counts/min due to [^{3}H]Δ^9-THC; open circles, protein in fractions (μg/ml).

In vivo experiments

The uptake of radioactivity by various tissues 1 h following intravenous injections of [^{3}H]Δ^9-THC in rabbits is given in Table I. The results are presented as tissue–plasma ratios (T/P) in order to eliminate individual variability due to varying concentrations of tracer in the plasma of the animals. The mean T/P ratio for choroid plexus of 1.69 was significantly different from, and in most cases more than two times higher than, other CNS tissues which ranged from 0.68 to 0.85.

DISCUSSION

The foregoing data indicate that the accumulation of [^{3}H]Δ^9-THC by choroidal epithelium and brain slices against concentration gradients has characteristics of active transport systems, *i.e.*, temperature dependence, metabolic inhibition, and saturable uptake components. Although the transport constants for the two tissues were remarkably close, indicating a similar enzyme affinity, values for both T/M and V_{max} were approximately 9-fold higher in choroid plexus than in brain cortex slices (Figs. 3 and 4). This represents a very high uptake for Δ^9-THC relative to values reported for other compounds by the choroid plexus[5]. Figs. 3 and 4 also demonstrate that active transport contributes strongly to Δ^9-THC uptake in both tissues at low media concentrations with appreciable non-saturable uptake at higher media concentrations that is probably due to diffusion. The latter component may be accounted for by the high affinity of Δ^9-THC for subcellular organelles (myelin,

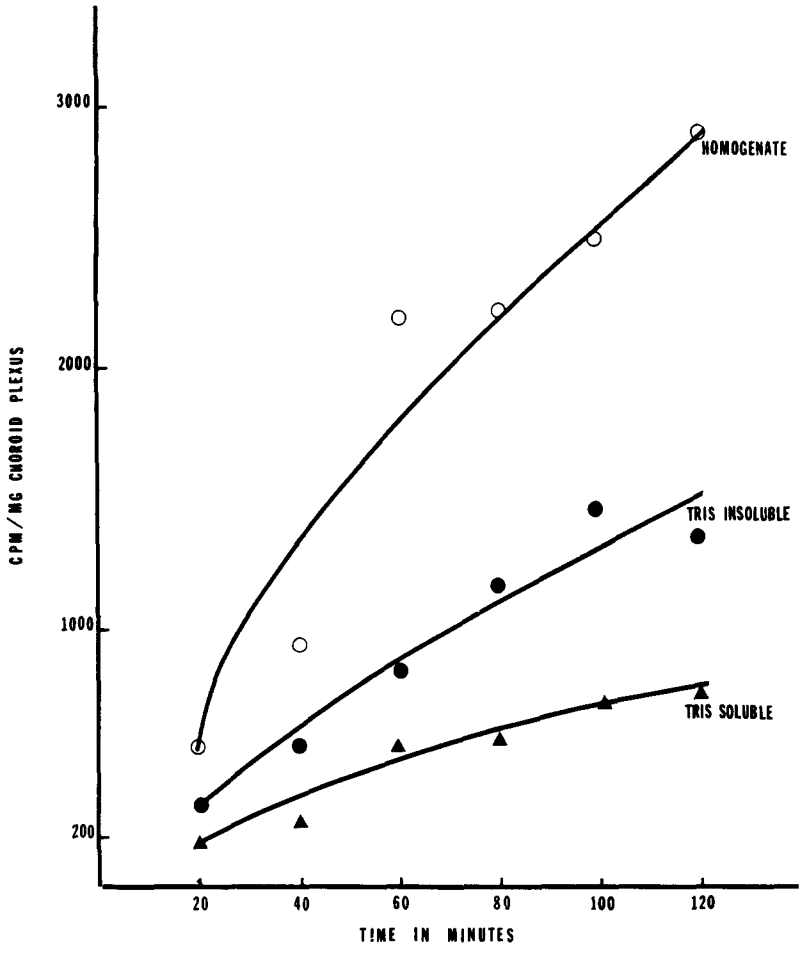

Fig. 6. Radioactivity in pH 7.4 Tris buffer homogenate fractions of choroid plexus incubated for 60 min in media containing 0.26 μCi/ml BSA–[^{3}H]Δ^9-THC suspension. Centrifugation was carried out at 12,000 $\times$ g for 20 min.

synaptosomes and mitochondria) which have been reported to passively accumulate this compound in rat brain homogenates[8]. Thus, the greater association of label with the Tris-insoluble fraction (containing these organelles), compared to the Tris-soluble (Fig. 6) is probably attributable to the lipophilic nature of Δ^9-THC as reported by previous workers[8,17]. In this regard a preferential localization of [^{3}H]Δ^9-THC in intracellular lipid inclusions of incubated rabbit choroid plexus epithelial cells has been demonstrated by autoradiography in this laboratory[1]. This supports evidence for the lipophilic nature of Δ^9-THC and may explain the relative irreversibility of choroidal, compared to brain uptake of [^{3}H]Δ^9-THC (Fig. 2). In addition, since Δ^9-THC was not metabolized in choroid plexus, its uptake by the lipid bodies is apparently due to physical processes once inside the cell.

The accumulation of Δ^9-THC by the rabbit choroid plexus *in vivo* was much

TABLE I

TISSUE DISTRIBUTION OF [^{3}H]Δ^9-THC ONE HOUR FOLLOWING INTRAVENOUS INJECTION IN RABBITS

Tissue	*T/P**	*P*
Choroid plexus	1.69 ± 0.28	
Parietal cortex	0.78 ± 0.08	< 0.005
Frontal cortex	0.74 ± 0.07	< 0.003
Midbrain	0.85 ± 0.08	< 0.010
Cerebellum	0.70 ± 0.06	< 0.003
Corpus callosum	0.68 ± 0.08	< 0.003
Liver	2.78 ± 0.13	
Kidney	2.47 ± 0.17	

* T/P = mean tissue–plasma ratios ± S.E. Each value represents the mean of 10 tissue determinations.

less than that observed *in vitro*, however, the 1-h distributions did indicate a selective uptake with respect to other regions of the brain. The lower concentrations of Δ^9-THC observed in the choroid plexus with an intact vascular supply, are not unexpected if transepithelial transport from CSF to blood occurs *in vivo*. Asghar and Way[3] demonstrated that only 25% of morphine extracted from cerebral ventricular perfusion media could be found in the whole brain or choroid plexus of rabbits following ventricular perfusions. The authors concluded that the remainder of the morphine was transported into the blood after its removal from the ventricular CSF by the choroid plexus. Considerable additional evidence exists for an excretory function of the choroid plexus which operates to maintain low levels of narcotics and other substances in the CSF[5,15,28]. Such a system operating for Δ^9-THC or other drugs has important implications for their regulation, not only within the CSF, but indirectly in brain by means of the CSF serving as a diffusional sink[6,10] and thereby limiting their access to the central nervous system. It is of interest that in addition to sharing choroidal transport characteristics of narcotics as presented above, Δ^9-THC also exhibits analgesic effects similar to that of morphine and codeine[20,21]. However, the milder anesthetic activity of Δ^9-THC is apparently more analogous to that of anesthetics such as trichloroethylene with which cannabis shares the property of high fat solubility[21].

The present results indicate that active transport processes are involved in the accumulation of Δ^9-THC in choroid plexus and to a less extent, in brain *in vitro* and suggest a participation in choroidal clearance of CSF *in vivo*. Clarification of the extent of net transport of Δ^9-THC from CSF to blood will require estimates by more quantitative techniques such as ventriculo-cisternal perfusions.

The clinical significance of the present results can only be speculative at this time, however the possibility of choroidal damage secondary to high concentrations of cannabis resulting in CSF secretory or reabsorptive dysfunction should be considered. Further studies on the uptake of Δ^9-THC in the presence of psychomimetic levels of cannabis and other hallucinogens are warranted.

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