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Δ^9-Tetrahydrocannabinol Transport in Rabbit Eyes

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The rabbit anterior uvea preparation in vitro accumulated Δ^9-tetrahydrocannabinol (THC). The concentrating mechanism was temperature and oxygen dependent and required glucose in the media. Dinitrophenol, iodoacetate, and cyanide inhibited THC accumulation. Ouabain, iodide, perchlorate, probenecid, iodipamide, and prostaglandin E_1 did not alter the accumulation ratio. Para-aminohippurate enhanced THC uptake. Following intravitreal injection [^{3}H]THC exited rapidly from the eye with a half-time of approximately 1·4 hr compared to a half-time of 15·8 hr for [^{14}C]sucrose.

Key words: Δ^9-tetrahydrocannabinol; ocular transport, in vivo, in vitro; rabbit; anterior uvea; intravitreal injection.

1. Introduction

Δ^9-Tetrahydrocannabinol (THC), the major active component of marijuana, is reported to lower intraocular pressure in man following smoking (Hepler, Frank and Ungerleider, 1972) or intravenous injection (Purnell and Gregg, 1975) and in rabbits following intravenous or topical administration (Green and Bowman, 1976). Rabbit irides (and ciliary processes) concentrate THC after topical administration (Green, Bigger, Kim and Bowman, 1977). The present study demonstrates the in vitro accumulation of THC by the rabbit anterior uvea and the in vivo transport of THC out of the rabbit eye.

2. Materials and Methods

Male albino rabbits weighing 1·5–2·5 kg were killed by intracardiac air injection. The eyes were promptly enucleated, opened posteriorly, and the lens and vitreous carefully dissected free. The entire anterior uvea was removed as a complete ring weighing approximately 45 mg. This tissue was placed in 2 ml of Tyrode's solution which had been bubbled with 5% CO_2–95% O_2 to adjust the pH to 7·4. [^{3}H]THC (New England Nuclear, specific activity 11·7 Ci/μmol) was evaporated to dryness and reconstituted in the Tyrode's media to 1·3 μCi/ml (31·2 ng/ml).

Incubations were carried out with gentle shaking in a water bath from 5 to 120 min. The tissues were then blotted on filter paper, weighed, and homogenized in 1 ml Tyrode's solution. The homogenate was centrifuged at 2500 rev/min. Supernatant (0·1 ml) and incubation media (0·1 ml) were counted in a liquid scintillation spectrometer (Packard Tri Carb, Model 3375). Correction for quenching was made by the external standard technique. The water content of the anterior uvea was found to be 85% by weighing before and after drying to constant weight. All values presented are corrected to a tissue water basis. The uptake of [^{3}H]THC was expressed as tissue–media (T/M) ratios:

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

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In saturation and inhibition experiments, nonlabeled THC (National Institute on Drug Abuse) was added to the medium prior to incubations. Solutions of metabolic inhibitors and organic compounds were prepared with Tyrode's as solvent and added to media to achieve final concentrations as indicated. In inhibition experiments the anterior uvea from one eye was incubated with the inhibitor while that from the other eye of the same rabbit served as a paired control and was incubated in media without inhibitor. In low sodium incubations the deficit of sodium in the media was compensated by equimolar concentrations of choline.

Thin layer chromatography was performed on methanol extracts of [^{3}H]THC stock solutions and on extracts of anterior uvea tissue incubated in Tyrode's solution containing [^{3}H]THC for 1 hr. A modified method was used with a 1:1 chloroform : acetone solvent system and silica gel plates (Ho, Estevez, Englert and McIsaac, 1972).

To determine the contribution of non-specific binding to total apparent [^{3}H]THC uptake, some specimens were weighed, homogenized and sonicated (Artek Sonic Dismembrator). The homogenate was sealed into 1 cm diameter Spectropore dialysis tubing with 1 ml of Tyrode's solution and incubated in 20 ml medium containing [^{3}H]THC for 6 hr. The sample and medium were each counted for radioactivity.

Intravitreal injections were performed on awake rabbits using topical proparacaine hydrochloride 0·5% anesthesia. A microsyringe (Hamilton 700 series) with a stop assembly (Chaney adaptor) was used. The eye was proptosed and the needle inserted approximately 3 mm posterior to the limbus. The needle was observed through the pupil in the mid-vitreous cavity. Tension of proptosis was released and 0·01 ml injected. The solution used was balanced salt solution to which was added 1·0 μCi/ml [^{3}H]THC and 1·0 μCi/ml [^{14}C]sucrose (New England Nuclear, 4·9 mCi/μmol). Ten rabbits received probenecid 50 mg/kg intraperitoneally 1 hr before intravitreal injections. In some experiments unlabeled THC 4·5 μg/ml was added to the above solution. The animals were killed by intracardiac air emboli at 0·5 to 6 hr after intravitreal injections. The injection site was frozen with dry ice, the eye proptosed, and aqueous humor removed. The eye was enucleated and immediately frozen in a mixture of acetone and dry ice. The sclera was incised posteriorly and retracted. The frozen vitreous body was removed and weighed. Radioactivity of the total vitreous body and 0·1 ml of aqueous humor was determined as described above using dual ^{3}H–^{14}C channel counting.

The microsyringe assembly had a repeatable accuracy of approximately $\pm$0·5%. Zero time radioactivity recovery:

$$\frac{\text{(ct/min)/vitreous body}}{\text{(ct/min)/0·01 ml injected solution}} \times 100$$

was 58% for [^{3}H]THC and 40% for [^{14}C]sucrose. The low recovery was due to quenching by the vitreous. This zero time percentage recovery was used to correct the amount of radioactivity observed at various times following intravitreal injections:

$$\text{Percent remaining} = \frac{\text{(ct/min)/vitreous body at designated time}}{\text{(ct/min)/0·01 ml injected solution} \times \text{zero minute recovery}} \times 100$$

Intravenous injection of 50 μCi [^{3}H]THC was performed in rabbits. Two or 4 hr later blood was withdrawn and the animals killed. Aqueous humor was obtained and the eyes removed. The vitreous body was removed after the globe was frozen. Radioactivity was determined in the plasma, aqueous humor, and vitreous humor.

3. Results

At 37°C the T/M values increased linearly after the first 10 min for periods up to 2 hr (Fig. 1). At 2°C, accumulation was almost abolished, the T/M ratio reaching 1·8 at 10 min and only 2·5 at 2 hr. In the dialysis experiment the disrupted tissue (sonicated homogenate) did not accumulate [^{3}H]THC.

Thin layer chromatography of anterior uveal preparations following 1 hr incubation with [^{3}H]THC gave a single peak with an R_f value of 0·60. This was identical to that observed in chromatography of the stock solution of the labeled compound.

Accumulation at 1 hr was reduced by 42% in the absence of oxygen (bubbling nitrogen through the media). Omitting glucose from the media decreased uptake of [^{3}H]THC by 38%. Substitution of potassium-free, calcium-free, or low sodium Tyrode's media did not alter the accumulation. Addition of increasing concentrations of non-labeled THC substrate caused a progressive decline in accumulation of radioactive label.

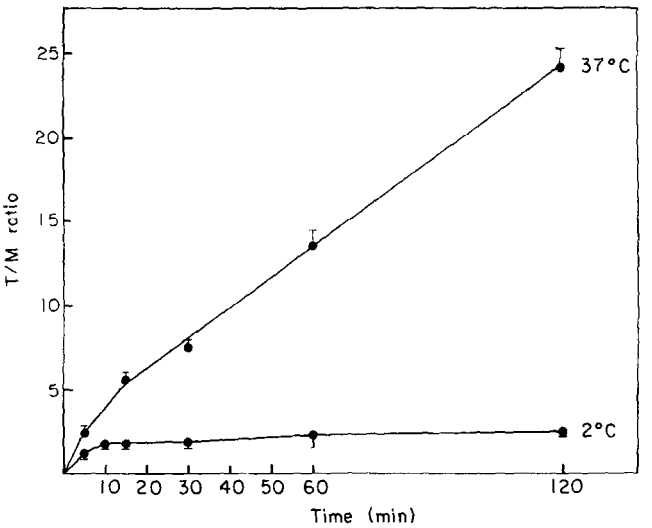

FIG. 1. Time course of accumulation of [^{3}H]THC by rabbit anterior uvea. Each point shows the mean of eight samples. Vertical lines indicate standard errors of the means.

TABLE 1

Effect of various inhibitors on [^{3}H]THC uptake by rabbit iris-ciliary body preparations (60 min incubation)

Inhibitors	[^{3}H]THC (T/M)* Control	Experimental	Percent Change†
Dinitrophenol (10^{-4} M)	13·92	7·14	−49
Iodacetate (10^{-3} M)	12·15	6·23	−49
Cyanide (5×10^{-4} M)	13·18	4·40	−67
Ouabain (5×10^{-3} M)	11·41	10·50	− 8
Probenecid (10^{-3} M)	11·87	13·28	+12
Iodide (10^{-3} M)	11·13	10·69	+ 4
Perchlorate (10^{-3} M)	16·30	16·08	+ 1
Para-aminohippurate (10^{-3} M)	11·94	18·10	+52
Iodipamide (10^{-3} M)	11·75	10·65	+ 9
Prostaglandin E_1 (10^{-3} M)	12·47	14·64	+17

* Each mean is based upon six to eight paired samples.

† % change $= \dfrac{\text{T/M control} - \text{T/M experimental}}{\text{T/M control}} \times 100.$

A variety of metabolic inhibitors added to the media significantly reduced the capacity of the tissue to concentrate THC (Table I). These included 2, 4-dinitrophenol (10^{-4} M), iodoacetate (10^{-3} M), and cyanide (5×10^{-4} M). However, the accumulation of [^{3}H]THC was not influenced significantly by the addition of probenecid, ouabain, iodide, perchlorate, iodipamide, or prostaglandin E_1 to the media. The presence of para-aminohippurate (10^{-3} M) significantly ($P < 0{\cdot}02$) increased [^{3}H]THC accumulation.

The time course for the exit from the eye of [^{3}H]THC and [^{14}C]sucrose following intravitreal injection in vivo is shown in Fig. 2. Sucrose disappeared from the vitreous body at its usual slow rate (half lost in 15·8 hr). The [^{3}H]THC disappeared very rapidly (half-time of 1·4 hr). Four hours following the intravitreal injection of [^{3}H]-THC 83% of the radioactivity was lost from the vitreous, but only 2% of the

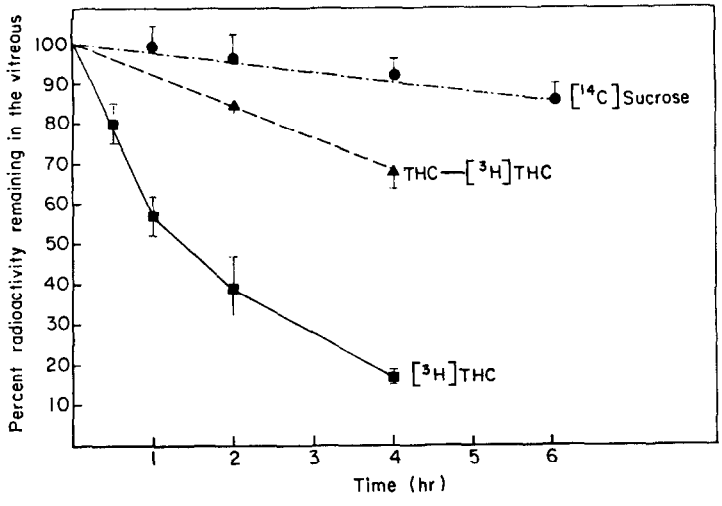

FIG. 2. Changes in ^{3}H- and ^{14}C- activities in the vitreous humor following intravitreal injection of a mixture of [^{3}H]THC and [^{14}C]sucrose with and without nonlabeled THC. Each point shows the mean of 10 samples. Vertical lines indicate standard errors of the means.

^{3}H activity originally injected into the vitreous was recovered from all of the intraocular tissues (ciliary body-iris, lens, retina and choroid). Systemic pretreatment with probenecid did not alter the exit time of either [^{3}H]THC or [^{14}C]sucrose. Following probenecid the percentage of THC remaining was 43·8 at 2 hr and 16·2 and 4 hr. The addition of nonlabeled THC (Fig. 2) delayed the loss of [^{3}H]THC from the eye, with 84·5% remaining at 2 hr and 68·2% at 4 hr (half-time of 6·3 hr). [^{3}H]THC was detected in the anterior chamber aqueous humor only after the addition of nonlabeled THC to the intravitreal injection solution.

Two hours following intravenous injection of [^{3}H]THC, radioactivity was detected in both the anterior chamber aqueous humor and vitreous. The tissue–plasma ratio was 0·41 for aqueous humor and 0·27 for vitreous.

4. Discussion

The rabbit anterior uvea is capable of accumulating ^{3}H-labeled THC against a concentration gradient. Accumulation is temperature dependent and requires glucose and oxygen. The effect of metabolic inhibitors and the absence of demonstrable non-

specific tissue binding is suggestive that the accumulation is active and probably carrier mediated. We were unable to calculate meaningful kinetics for this system due to THC's limited solubility in water (5 μg/ml) (Paton and Pertwee, 1973). Anterior uveal uptake is similar to that reported for choroid plexus (Agnew, Rumbaugh and Cheng, 1976). THC accumulation by the anterior uvea is of interest since this agent may lower intraocular pressure by decreasing the rate of aqueous humor production.

Thin layer chromatography indicates no metabolism of [^{3}H]THC by the anterior uveal in vitro. This is in agreement with previous observations of the absence of negligible metabolism of THC by brain tissue in vitro (Christensen et al., 1971).

The transport of THC by the anterior uvea is not sensitive to ouabain. This has also been reported for the choroid plexus (Agnew, Rumbaugh and Cheng, 1976). In addition, the lack of dependence on sodium or potassium in the media suggests that Na-K ATPase activity is not important to THC transport kinetics.

The anterior uvea can actively accumulate a variety of anions and organic acids. At least three different anion transport systems have been previously characterized. On the basis of the most commonly used test substances and/or inhibitors, these transport systems can be referred to as the iodide (Becker, 1961), the PAH (Barany and Kinsey, 1949) and the iodipamide (Barany, 1973) systems. The fact that neither perchlorate or iodide nor iodipamide have an effect on THC accumulation suggests that the "iodide system" and the "liver-like" or "L system" are not involved. Prostaglandin transport represents a subcomponent of the iodipamide system (Bito, 1972). Prostaglandin E_1 has no effect on anterior uveal THC transport. The addition of PAH to the media increases THC accumulation. Stimulation of organic cation transport by addition of another organic cation has been described for renal tissue (Hewitt, Clark and Hook, 1976). Further studies are being performed concerning this enhancement in anterior uveal preparations.

Intravenously administered [^{3}H]THC enters both the vitreous and aqueous humor across the blood–ocular barrier. Once within the eye, THC appears to be removed rapidly without significant metabolism. Intravitreal THC is lost much faster than sucrose. This implies active transport as the molecular weights of THC (342) and sucrose (354) are nearly identical and their diffusion rates should be similar. Essentially all of the THC which is lost from the vitreous compartment actually leaves the globe. The exit site must be posterior to the lens for at low concentrations no THC enters the anterior chamber. At higher concentrations THC does reach the anterior chamber, implying that the in vivo transport is saturable, much as is the separate prostaglandin removal system (Bito and Salvador, 1972).

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