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The non-specific membrane binding properties of Δ^9-tetrahydrocannabinol and the effects of various solubilizers[1]

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The binding of [^{3}H]Δ^9-tetrahydrocannabinol to crude and purified synaptosomal membrane suspended in either Krebs solution or 10mM sodium phosphate buffer was examined. The membrane/buffer partition coefficient was found to be 12 500, and was constant over a free concentration range of 10^{-8} to 10^{-6}M. Binding was similar in both suspending media, and to both crude and purified synaptosomal membrane. The solubilizing agent Cremophor E.L. (8 μg ml^{-1}) decreased the partition coefficient by one-half, and by greater than 99% at 0·4 mg ml^{-1}. Similar effects were observed with Tween 80, while ethanol caused a maximum decrease of 60%. Membrane concentrations of THC were calculated at various effective concentrations reported in the literature, and were within the range predicted by the Meyer-Overton rule of anaesthesia. An apparent non-specific interaction with neuronal membranes and effective membrane concentrations of the order 2×10^{-4} to 1×10^{-2} mol kg^{-1} suggests THC may exert some of its effects by a mechanism analogous to the general anaesthetics, and thus may be classified as a partial anaesthetic.

The cellular or molecular mechanism of action of Δ^9-tetrahydrocannabinol (THC) has not been well established. The many pharmacological effects observed in vivo and in vitro suggest that THC may combine with a specific receptor or selective membrane component(s) (Seeman et al 1972) while other data suggest that it may interact non-specifically with neuronal membrane in a manner analogous to the anaesthetics (Mahoney & Harris 1972; Paton et al 1972; Lawrence & Gill 1975). THC is a very lipid soluble molecule, with a relatively high octanol: water partition coefficient (Gill & Jones 1972; Roth & Williams 1977), which suggests a possible interaction with hydrophobic regions in the membrane (Mahoney & Harris 1972), a common characteristic of many anaesthetics (Roth & Seeman 1971). THC, however, is apparently incapable of producing surgical anaesthesia in animals and has thus been termed a partial anaesthetic (Paton et al 1972; Lawrence & Gill 1975). Many other of its actions support the hypothesis that THC is anaesthetic-like. To determine whether THC interacts with cellular components (i.e. neuronal membrane) non-specifically, and to evaluate whether the binding characteristics at effective concentrations are consistent with the lipid solubility rule of anaesthesia (Roth & Seeman 1972) the binding properties of THC were examined on crude and purified synaptosomal membrane. Adsorption isotherms for THC (Seeman et al 1972), showed a decrease in partition coefficient with increase in extracellular concentration, suggesting the presence of specific cannabinoid receptors. However, the concentrations used in that study were in the upper range effective in in vitro experiments and are far removed from the concentrations suggested for in vivo effects (Gill & Lawrence 1974). Those concentrations also may have exceeded the solubility of the drug (Garrett & Hunt 1974). Since various solubilizing agents or solvents have often been used to overcome the low aqueous solubility of THC (Acosta-Urquidi & Chase 1975; Banerjee et al 1975), the effecs of three commonly used solubilizers, Cremophor E.L. (Roth 1976), Tween 80 (Graham et al 1974) and ethanol (Alhanty & Livne 1974) on the membrane binding were also examined. A portion of this study has been previously reported (Roth & Williams 1977).

METHODS

Membrane suspensions

Brain membrane suspensions were prepared essentially as described by Whittaker et al (1964). Adult

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male Sprague-Dawley rats (350–700 g) (Medical Vivarium, University of Calgary) were decapitated (Harvard apparatus) and the brain rapidly dissected over ice, chopped roughly, placed into 10 volumes of ice cold sucrose (0·32 M), and homogenized with 10 strokes of a Teflon/glass tissue homogenizer. The homogenate was transferred to tubes (50 ml Oak Ridge polycarbonate) and centrifuged at 1000 *g* (10 min). The supernatant was removed with a glass pipette, and recentrifuged at 10 000 *g* (30 min). The resulting pellet (P_2) was resuspended in either Krebs solution or 10mM phosphate buffer. Complete washing of the pellet was by three successive re-centrifuging and re-suspending steps. The final suspension was stored in polycarbonate tubes at 4 °C for use, usually within 3 to 4 days. The dry weight of the membrane suspension was determined by lyophyllizing weighed samples and corrections were made for dry weight of salts. Dry weight was calculated as mg dry weight membrane ml^{-1} of suspension.

The P_2 fraction (crude synaptosomal membrane) was further purified by layering aliquots of suspension onto a discontinuous sucrose density gradient (0·8 M/1·2 M sucrose), and centrifuging at 50 000 *g* (2 h). The fraction at the interface was removed and resuspended in a similar manner to the P_2 fraction. This fraction represents the purified synaptosome fraction of brain membranes.

Solutions

Suspending media consisted of either Krebs solution or 10 mM phosphate buffer. Binding data have usually been obtained using phosphate buffer (Roth & Seeman 1972), however binding in the presence of Krebs solution was examined for comparison with published in vitro experiments. The Krebs solution had the following composition (mM) NaCl 95; KCl, 4·7; $MgSO_4$, 2·3; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $NaHCO_3$, 25; dextrose, 12.

Radiolabelled drug solutions

^{3}H-THC and unlabelled THC were generously supplied by the Health and Welfare Department, Canada, through the offices of Mr R. G. Graham. Stock solutions were prepared in absolute ethanol, and stored in glass volumetric flasks kept in the dark at 4 °C. Incubation solutions were prepared by placing a measured aliquot of labelled and unlabelled THC (if necessary) in a glass vial, and evaporating to dryness under nitrogen. The resultant residue was suspended in Krebs solution or phosphate buffer.

Binding procedure

A 2 ml aliquot of drug solution was added to a 10 × 75 mm Pyrex tube with an Eppendorf pipette, followed by a 0·5 ml aliquot of membrane suspension. A control tube was prepared for every 3 sample tubes and was treated identically except that an equal volume of physiological solution replaced the membrane suspension. The tubes were vortexed for 10–15 s, and then allowed to stand for 30 min at room temperature (20 °C). Approximately every 10 min during this incubation a 10 s pulse on the Vortex mixer was repeated. At the end of the incubation, the tubes were centrifuged at 20 000 *g* at room temperature for 15 min. Triplicate aliquots of 0·5 ml of the supernatant were taken using Eppendorf pipettes and placed into 20 ml glass scintillation vials for counting. A 10 ml aliquot of scintillation fluid (Aquasol-New England Nuclear or ACS-Amersham Searle) was added to each vial and the solution was counted to less than 1% error on either a Packard TriCarb or Searle Mk III Scintillation counter. Counts were converted to disintegrations min^{-1} (d min^{-1}) using the sample channels ratio (SCR) method (Packard TriCarb) or automatic external standard (AES) ratio (Searle).

Calculations

The methods used for calculating the amount of drug bound to membrane were adapted from those of Roth & Seeman 1972 and Seeman et al 1972 and were based on a comparison between the final free concentration in the control tube (without membrane) and the supernatant in the sample tube (containing membrane). The difference between the two represents the amount of drug adsorbed to the membrane. A major assumption is that the total amount of drug available for binding is identical in both tubes. To test this, a series of tubes were prepared containing identical aliquots of drug solution, and varying weights of membrane. Physiological solution in appropriate aliquots was used to replace membrane suspension. The tubes were mixed, and samples were taken from each after a 30 min incubation as described, and then counted. The total drug (i.e. radioactivity) in suspension was determined and plotted as a function of membrane dry weight in each tube. The increase in available drug in the tubes containing membrane (see Results) was used as a correction for the controls. Membrane concentrations are expressed as molality (mol kg^{-1} dry membrane—C_m) and free concentration in terms of molarity (mol $litre^{-1}$–C_f). The ratio of

membrane and free concentrations is the partition coefficient—P.

RESULTS

A. *Correction for total available drug*

The amount of drug in the suspension before centrifugation was dependent on the amount of membrane present (Fig. 1). THC binds extensively to glassware (Garrett & Hunt 1974), this binding is related to the free concentration of THC in solution which is in equilibrium with the THC bound to the glass. Thus

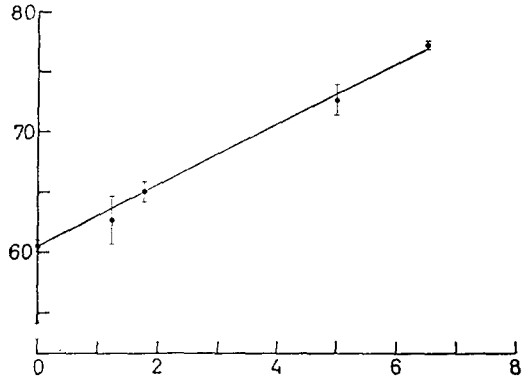

FIG. 1. Total d min^{-1} (^{3}H-THC) in suspension is plotted against dry weight of synaptosomal membrane in the tube. Each point is the mean (± s.e.m.) of at least 3 determinations. The total free concentration of THC is linearly dependent on amount of membrane in the tube. Ordinate: total d $min^{-1} \times 10^3$ in suspension. Abscissa: kg dry membrane/tube $\times 10^{-7}$.

the membrane, by decreasing the free concentration in the tube, will effectively decrease the amount of THC bound to the glassware. A comparison of radioactivity in the control tube with the supernatant of the sample tube without a correction for the total drug concentration available for binding, would result in a low value for membrane concentration. Corrections were thus made by adding an amount, calculated on the basis of total drug added and amount of membrane present, to the membrane concentration calculated for each tube. This led to an increase in the value of P of approximately 20%.

The addition of solubilizer, especially that of Cremophor E.L. to the physiological solution decreased binding to the glass. No dependence of total available drug on membrane weight was observed. No correction was, therefore, applied to such results.

B. *Membrane binding of THC*

The data for the adsorption of THC to the P_2 fraction suspended in Krebs solution were plotted using the double reciprocal method and there was a linear relationship between the reciprocals of membrane concentration, C_m^{-1}, and free concentration C_f^{-1}, (Fig. 2) ($r = 0{\cdot}97$) indicating the presence of only one binding site over a range of approximately 10^{-8} to 10^{-6} M. The partition coefficient (P) calculated from the slope of the regression line is 12 586 indicating that the membrane has an extremely high affinity for THC. The maximum binding to the membrane calculated from the intercept, is of the order of 16·8 mmol kg^{-1} dry membrane, which is similar to that

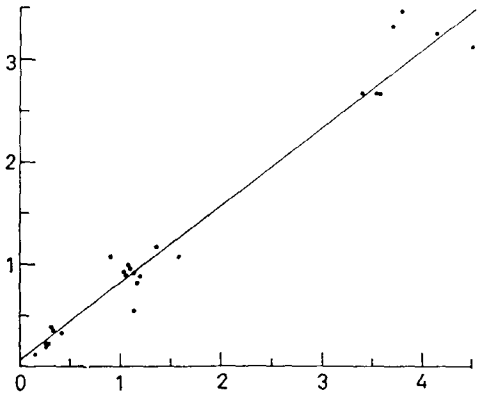

FIG. 2. Adsorption isotherm for THC to crude synaptosomal membrane plotted by the double reciprocal method. The reciprocal of the membrane concentration (C_m^{-1}) is linearly related ($r = 0{\cdot}97$) to the reciprocal of the free concentration (C_f^{-1}) over the concentration range studied. Each point is the mean of 3 determinations. Partition coefficient (P) calculated from the slope of the regression line is 12 586, and the maximum binding to the membrane calculated from the Y intercept is of the order of 16·6 mmolal. Ordinate: $C_m^{-1} \times 10^3$. Abscissa: $C_f^{-1} \times 10^7$.

found for a variety of non-specific lipid soluble anaesthetics (Roth & Seeman 1972). The data plotted by the Scatchard plot (P vs C_m), often used to demonstrate the presence of multiple binding sites (Seeman et al 1971), show the value of P to be constant indicating the presence of only one binding site (Fig. 3). The mean value for P is 12 477 (s.d. 2092). The result for the P_2 fraction in phosphate buffer gave a P value of 12 100 (s.d. 2432).

The results for the binding of THC to the purified synaptosomal membrane fraction suspended in Krebs solution are essentially similar to those for the P_2 fraction. The double reciprocal plot shows a linear relationship with a value of P, calculated from the slope, of 14 089 and the maximum solubility or binding to the membrane of the order of 4 mmol kg^{-1} dry weight membrane. The Scatchard plot also shows

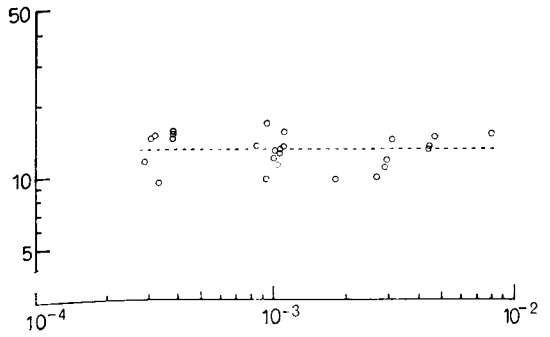

FIG. 3. The binding data of Fig. 2 are plotted by the Scatchard method (P vs C_m). The dotted line indicates the calculated mean value for P (12 477 ± 232). The value of P remains constant over the concentration range studied indicating a non-specific interaction for THC. Ordinate: P × 10^3. Abscissa: C_m.

that P is constant over the concentrations studied, the mean value for P is 11 426 (s.d. 2 409).

C. Effects of solubilizers on the membrane binding of THC

Since THC is almost insoluble in water, solubilizers have been used. The effects of three of them, Cremophor E.L., Tween 80, and ethanol, on the membrane binding of THC were examined.

Cremophor E.L. Membrane binding is drastically reduced by Cremophor E.L. (Fig. 4) At concentrations of the solubilizer as low as 8 μg ml^{-1}, P is decreased by 50%. At a concentration of 0·4 mg ml^{-1}, P is decreased to 1/200th of its orginal value. A graph of P *vs* THC concentration in the presence of 3 concentrations of the solubilizer shows a linear relationship between P and C_f, and P remains constant over a wide range of free concentrations, (Fig. 5) which again suggests the presence of a single population of binding sites.

Tween 80. The effects of Tween 80 are qualitatively similar to those of Cremophor E.L., the curve being almost superimposable on the Cremophor curve. At 10 μg ml^{-1}, the solubilizer was able to decrease the binding of THC to 1/3 of its control value.

Ethanol. The effects of ethanol on the binding of THC were not similar to the effects of the other two solubilizers. The data for the effects of 4 concentrations of ethanol on P for THC are shown in Table 1. P was decreased to a lesser extent by ethanol than by Cremophor E.L. or Tween 80. The effect reached a plateau at a relatively high value, i.e. approximately 45% of original. It appears that ethanol does not appreciably increase the solubility of the drug (Garrett & Hunt 1974).

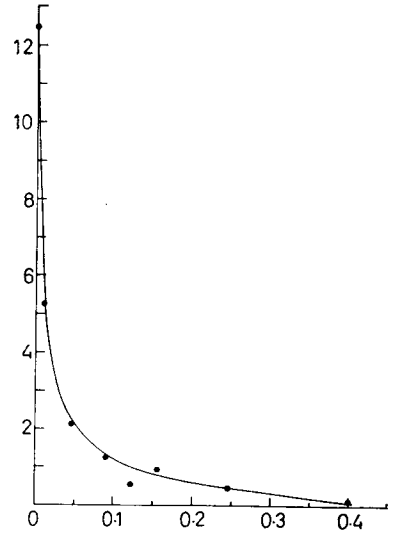

FIG. 4. The partition coefficient (P) of THC in the presence of varying concentrations of Cremophor E.L. Each point is the mean of at least 15 determinations. s.e.m. values (not shown) are on average 5·4% of the mean. Ordinate: P × 10^{-3}. Abscissa: Cremophor E.L. (mg ml^{-1}).

D. Aqueous solubility of THC

Some reports have emphasized the relatively low aqueous solubility of THC. Some of these values are presented in Table 2.

During the course of our experiments the maximum concentration found in solution after centrifugation at 20 000 *g* was 9·5 ± 0·4 × 10^{-7} M (final concentration) where the initial solution prepared contained 3·2 × 10^{-4} M. The concentrations did not exceed the aqueous solubility of THC emphasizing that the upper limit of aqueous concentration of

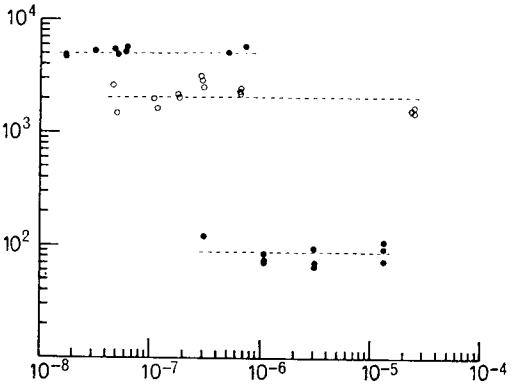

FIG. 5. Partition coefficient (Ordinate: P) is independent of the free concentration of THC but varies with concentration of Cremophor E.L. Partitions in the presence of 8, 40 and 400 μg ml^{-1} Cremophor E.L. are shown in descending order. Dotted lines indicate mean P values. Abscissa: THC (M).

Table 1. Effect of ethanol on partition coefficient (P) of THC.

Ethanol concn (% v/v)	P	s.e.m.	n
0·0	12477	±232	81
0·5	7442	±268	27
1·0	7647	±288	27
2·0	5852	±148	22
5·0	5829	±146	12

THC that can be used in in vitro experiments is of the order of 10^{-6} M.

E. *Octanol/water-membrane/buffer partition coefficients*

A correlation between membrane/buffer (P) and octanol/water ($P_{o/w}$) partition coefficients for a series of lipid soluble anaesthetics has been shown (Machleidt et al 1972). On the average, $P_{o/w}$ is 5 fold greater than P. If the correlation could be extended to include THC, then from the value of P, a $P_{o/w}$ of the order of 60 000 is predictable which is in sharp contrast to a previously published value of 6000 (Gill & Jones 1972).

A recent report by Leo et al (1976) demonstrated correlations between log $P_{o/w}$ and molecular volume calculated from CPK molecular models. A molecular model of THC (Ealing CPK Atomic Models) was constructed and its volume determined by displacement of water. A calculation of the molecular volume for THC was also made using the method of Bondi (1964). The volumes calculated were 154·2 cm^3 mol^{-1} by the method of Leo et al and 170·4 cm^3 mol^{-1} by the Bondi method. Substitution of these values into the equations of Leo et al gave the values for log P shown in Table 3. These are only approximate because of the uncertainty in the assignment of THC to a particular group of compounds. The results suggest the $P_{o/w}$ may be in the range of 8×10^4 to 2×10^8, i.e. in excess of 6000. A value of approximately 60 000 calculated on the basis of the P does not appear unreasonable.

Table 2. Solubility of THC in vaious aqueous media.

Medium	Solubilizer	Maximum solubility (M)	Reference
Krebs	†	$1·53 \times 10^{-6}$	Jakubovic & McGeer (1972)
Saline	5% ethanol	$6·7 \times 10^{-5}$	Garrett & Hunt (1974)
Saline	†	$3·3 \times 10^{-6}$	Garrett & Hunt (1974)
Krebs	1% DMSO	$6·3 \times 10^{-7}$	Banerjee et al (1975)
0·9% saline	0·37% PVP	$3·9 \times 10^{-6}$	Johnson et al (1976a)
0·15M NaCl	†	$4·0 \times 10^{-6}$	Bach et al (1976)

† None quoted.

F. *Membrane concentration*

The Meyer Overton rule of anaesthesia predicts that anaesthesia occurs when the membrane concentration reaches 20 to 30 mmol of drug kg^{-1} dry membrane (Roth & Seeman 1972). Local anaesthesia occurs at this concentration, while for general anaesthesia the predicted concentration would be of the order of 2–3 mmolal. Since THC has been referred to as a partial anaesthetic and since its binding characteristics suggest a non-specific mode of action analogous to the anaesthetics, whether THC achieves, at effective concentrations, a membrane concentration predicted by the lipid solubility rule was determined.

Table 3. Octanol water partition coefficients ($P_{o/w}$) calculated for THC using equations described for log P/ molecular volume relationships*.

Group	Calculated Log $P_{o/w}$	$P_{o/w}$	Eqn*
Alkane	5·39†	$2·45 \times 10^5$	2a
	8·28	$1·95 \times 10^8$	3a
Haloaromatic	7·47	$2·95 \times 10^7$	3b
Substituted aromatic	4·91†	$8·13 \times 10^4$	2b
	6·09	$1·23 \times 10^6$	4
Alcohol	5·10	$1·26 \times 10^5$	5
Substituted phenol	5·37	$2·34 \times 10^5$	6
Haloalkane	6·98	$9·55 \times 10^6$	7

* Refers to equations described by Leo et al (1976).
† Log P calculated using molecular volumes by the method of Bondi (1964).

Calculations of C_m were made on the basis of published reports of effective concentrations (C_f) where: $C_m = P \times C_f$.

In studies where no solubilizer was used, values for P were based on those found in this study. The effects of Pluronic F68 and polyvinylpyrrolidone (PVP) on P were assumed to be similar to Tween 80 or Cremophor E.L. DMSO appeared to have little effect on the solubility of THC (Banerjee et al 1975) therefore its effects were regarded as negligible. The results of the calculations are shown in Table 4.

DISCUSSION

The binding of THC to crude (P_2) and purified synaptosomal membrane yields a partition coefficient of about 12 500. The binding characteristics in the presence of either 10 mM sodium phosphate

Table 4. Calculated membrane concentrations (C_m) at effective free concentrations (C_f) for various test systems.

Preparation	Measured response	Cf (M)	Response % of control	Solubilizer	P*	Cm*	Reference
Guinea-pig ileum	Contraction	$3{\cdot}2 \times 10^{-6}$	—	—	12 500	$4{\cdot}0 \times 10^{-2}$	Gill et al (1970)
Guinea-pig ileum	Contraction	$1{\cdot}6 \times 10^{-7}$	50	—	12 500	$2{\cdot}0 \times 10^{-3}$	Layman & Milton (1971)
Rabbit vagus	Action potential depression	$5{\cdot}0 \times 10^{-4}$	17	Pluronic F-68 1 mg ml^{-1}	100	$5{\cdot}0 \times 10^{-2}$	Byck & Ritchie (1973)
Rat vas deferens	Noradrenaline release	$5{\cdot}6 \times 10^{-7}$	−50	Tween 80 0·025 mg ml^{-1}	3000	$1{\cdot}68 \times 10^{-3}$	Graham et al (1974)
Mouse brain Synaptosomes	Uptake of dopamine	$5{\cdot}4 \times 10^{-6}$	50	Ethanol 1%	7647	$4{\cdot}0 \times 10^{-2}$	Howes & Osgood (1974)
Rat diaphragm	Twitch response	$3{\cdot}0 \times 10^{-5}$	50	Tween 80 10 mg ml^{-1}	100	$3{\cdot}0 \times 10^{-3}$	Kayaalp et al (1974)
Rat brain synaptosomes	Noradrenaline 5-HT uptake	$6{\cdot}3 \times 10^{-7}$†	65	DMSO 1% v/v	12 500	$7{\cdot}9 \times 10^{-3}$	Banerjee et al (1975)
Rat brain synaptosomes	5-HT uptake	$3{\cdot}9 \times 10^{-6}$†	50	PVP 3·7 mg ml^{-1}	500	$1{\cdot}95 \times 10^{-3}$	Johnson et al (1976a)
Rat brain synaptosomes	Noradrenaline uptake	$3{\cdot}9 \times 10^{-6}$	50	PVP 2·5 mg ml	500	$1{\cdot}95 \times 10^{-3}$	Johnson et al (1976b)
Lysolecithin acyl transferase	Enzyme inhibition	$3{\cdot}5 \times 10^{-7}$	50	DMSO 0·5%	12 500	$4{\cdot}4 \times 10^{-3}$	Greenberg et al (1977)
Guinea-pig ileum	Contraction	$1{\cdot}25 \times 10^{-7}$	50	Cremophor E.L. 0·01 mg ml^{-1}	1000	$1{\cdot}25 \times 10^{-4}$	Roth (1978)

* Calculated.
† Maximum solubility of THC quoted by authors.

buffer or Krebs solution are of the same order of magnitude. The determination of the membrane binding of THC is complicated by several problems primarily associated with the physicochemical properties of the drug, i.e. its low aqueous solubility (see Table 2), and high affinity for glassware (Garrett & Hunt 1974). Our results emphasize that the upper limit of aqueous concentration of THC obtained after centrifugation is of the order of 10^{-6} M or less.

The total amount of THC available for binding to membrane varied with the amount of membrane material present, and thus a correction factor to accurately determine actual membrane concentrations was needed. Appropriate correction of control values increased the apparent P by approximately 20%. Replacing the centrifugation method of separating bound from free drug with a filtration technique met with little success. THC binds to both polymeric (Metricel GA-7) and glassfibre (Whatman GF/C) filter membranes extensively, and total amounts bound to filters varied from experiment to experiment. Therefore, the centrifugation methods we used to measure membrane concentrations and P, while not ideal, still appear to be the most accurate if appropriate corrections are made for control values.

The adsorption isotherms of THC indicate that P remains constant over the range (C_f) 10^{-8} to 10^{-6} M suggesting that within this range the binding of THC to membrane is non-specific, and only one type of binding site (i.e. hydrophobic region) is available. The range used was limited by the maximum solubility of the THC in the aqueous media, and the specific activity of the radiolabelled compound available but it included those concentrations generally found to be effective in a variety of in vitro studies (see Table 4), and corresponds to membrane concentrations of 10^{-4} to 10^{-2} molal. From data of Gill & Lawrence (1974), brain membrane concentrations at effective doses for in vivo behavioural effects would be approximately 2×10^{-5} molal. Differences in membrane concentration are determined not only by the physicochemical properties of the drug, but also by the composition or nature of the membrane phase (Miller & Pang 1976).

The value we obtained for P contrasts with values reported previously (Seeman et al 1972; Paton et al 1972). Partition coefficients for THC reported by Seeman et al (1972) were around 800 decreasing to 350 for human erythrocyte ghost membranes, and approximately 500 decreasing to 45 for guinea-pig synaptosomes over a free concentration range of 4×10^{-6} to 4×10^{-5} M. It was also suggested that there could be two distinct binding sites for THC within synaptosomes. However, the concentration range indicated for C_f exceeded the aqueous solubility of THC. The value reported by Paton et al (1972) had been calculated on the basis of a rule previously established (Machleidt et al 1972), i.e. the membrane/buffer partition coefficient of an uncharged drug molecule is approximately one-fifth of the value of the octanol/water partition coefficient.

An accurate estimate of the $P_{o/w}$ for THC has not been reported, and in agreement with Gill et al (1970) we found this difficult. However, a method for calculation of $P_{o/w}$ has been described by Leo et al (1976) and by substituting the molecular volume of THC into equations for chemical classes thought

appropriate for THC gave values for log $P_{o/w}$ that ranged from 4·91 to 8·28, and therefore the predicted values for P would be of the order $1{\cdot}64 \times 10^4$ to $3{\cdot}8 \times 10^7$ which support the value of 12 500 determined in this study.

Solubilizing agents exert profound effects on the membrane binding of THC. Concentrations of Tween 80 and Cremophor E.L. used in in vitro experiments can decrease P by a factor of 100. The effects of ethanol are relatively small in comparison, it has only a slight effect on the aqueous solubility of THC (Garrett & Hunt 1974). THC and solubilizer may interact to form complexes (e.g. micelles) which effectively decrease the total availability of THC for binding. Or the solubilizers themselves being capable of interacting with the membrane as suggested by the observation that they can perturb excitable membrane (Roth, unpublished data), may effectively decrease the number and/or accessibility of sites available for THC.

The use of solubilizing agents to increase the aqueous solubility of THC does not increase the membrane concentration above that which would occur in the absence of a solubilizer at the same free concentrations (C_f). At a C_f of 3×10^{-7} M, the membrane concentration is $3{\cdot}7 \times 10^{-3}$ molal in the absence of solubilizer, and in the presence of 0·4 mg ml^{-1} Cremophor E.L. ($C_f = 3 \times 10^{-7}$ M) the membrane concentration is $3{\cdot}7 \times 10^{-5}$; a decrease of 100 fold, as illustrated by the decrease of P. The only apparent advantage of adding a solubilizer is to decrease the loss of THC to glassware and other apparatus.

The values for P in the presence and absence of solubilizers have enabled calculations of membrane concentrations to be made at effective concentrations reported in the literature. The results (Table 4) suggest that the effective membrane concentration of THC is of the order $1{\cdot}25 \times 10^{-4}$ to 4×10^{-2} molal which is well within the range predicted by the Meyer-Overton rule of anaesthesia (Roth & Seeman 1972). In vitro studies suggest that the mode of action may be analogous to anaesthetics (Paton 1975) and our binding data support this concept of a non-specific interaction. Many of the effects of THC may be explained in terms of a similar mode of action to anaesthetics, and thus the classification of THC as a 'partial anaesthetic' is supported.

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