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N-ETHYL-17(*R,S*)-METHYL-(6a*R*,10a*R*)-Δ^8-TETRAHYDROCANNABINOL-18-OIC AMIDE

BRAIN PHARMACOKINETICS IN MICE, TRIGLYCERIDE/PHOSPHOLIPID PARTITIONING AND GENERALIZATION TO THE DISCRIMINATIVE STIMULUS PROPERTIES OF Δ^9-THC IN RATS

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Abstract—The title compound, designed as a model for the affinity moiety of a cannabinoid affinity gel was synthesized in tritiated form (sp. act. 7.27 mCi/mmole). To validate the affinity approach to isolate the putative THC receptor, the properties of the amide were studied. Upon i.p. injection in mice the amide reaches peak brain levels of 0.13% of the total dose after 15 min. Following i.v. injection, maximal brain concentrations of 1.9% are observed at 5 min. Compared to Δ^9-THC, which distributes almost equally between triglyceride and phospholipid phases (51:49) the amide exhibits a strong preference for phospholipids (5:95) that can be interpreted as high relative membrane affinity. In rats trained in a water maze to discriminate between i.p. injections of 3 mg/kg Δ^9-THC (ED_{50} = 1.8 mg/kg) and its vehicle, the amide was generalized to the training drug, being five times less potent (ED_{50} = 8.7 mg/kg) than Δ^9-THC. This demonstration of cannabis-like activity indicates that the amide retains affinity to the postulated receptor and justifies the choice for the affinity ligand.

The psychotropically active cannabinoids Δ^9-THC and Δ^8-THC probably exert their action via a specific neuronal receptor, the "THC receptor" [1–3]. In order to characterize this receptor, or more generally, Δ^9- (or Δ^8-)THC binding neuronal macromolecules, an affinity chromatography approach was devised. Based on structure–activity relationships in the cannabinoid series and on model considerations 17(*R,S*)-methyl-Δ^8-THC-18-oic acid **2** was synthesized as a potential affinity ligand [4]. Coupling acid **2** to a commercially available aminohexyl-Sepharose [2] leads to affinity gels of low coupling yield. At present, a number of gels with an aminoethyl spacer group are being prepared by reacting BrCN activated Sepharose with ethylenediamine followed by coupling acid **2** via its *N*-hydroxy-succinimide ester **3** to the amino group of the spacer. A section of the basic structure of the affinity gel is given in Fig. 1.

The model compound closest to the final affinity unit is represented by the *N*-ethyl amide of acid **2**. Cannabis-like activity of this compound or, in our interpretation, affinity to the THC receptor was tested. In animal systems used for testing cannabis-like activity, relative potencies of the tested compounds are usually expressed as dosages at which half of the *Ss* show the drug adequate response. Their dosages (ED_{50}), however, are no measure of "relative receptor affinities" that could rather be expressed by the ratio of brain concentrations of the compound in question and its ED_{50}. Pharmacokinetics in turn are influenced by a number of factors, among them the partitioning of the compound between biological phases, e.g. the phospholipid phase of membranes, the triglyceride phase of adipose tissue and aqueous phases.

This paper reports the brain pharmacokinetics of amide **4** in mice after i.v. and i.p. injections of the drug, the *in vitro* triglyceride/phospholipid partitioning of **4** and the generalization of the discriminative stimulus properties of the amide to Δ^9-THC in rats. This information taken together gives a good indication of whether the affinity chromatography approach using acid **2** as ligand could be successful.

MATERIALS AND METHODS

1. *Synthesis of* [14,15-^{3}H-N]-*ethyl*-17(R,S)-*methyl*-Δ^8-*THC*-18-*oic amide* **4**. Since the synthesis of the tritiated ester **1** [5] and the preparation of the nontritiated amide **4** [6] have been reported in detail, the synthesis of the labelled amide **4** will be summarized briefly (cf. Scheme 1, overleaf).

Acid **2**, obtained from the tritiated ester **1** by alkaline hydrolysis (KOH in H_2O/methanol) in 92%

* Deceased on 15 February, 1984.

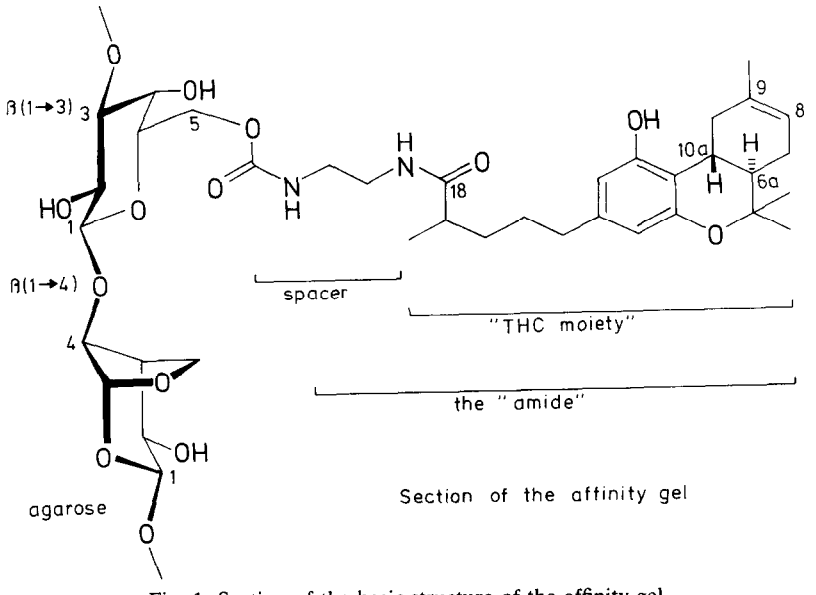
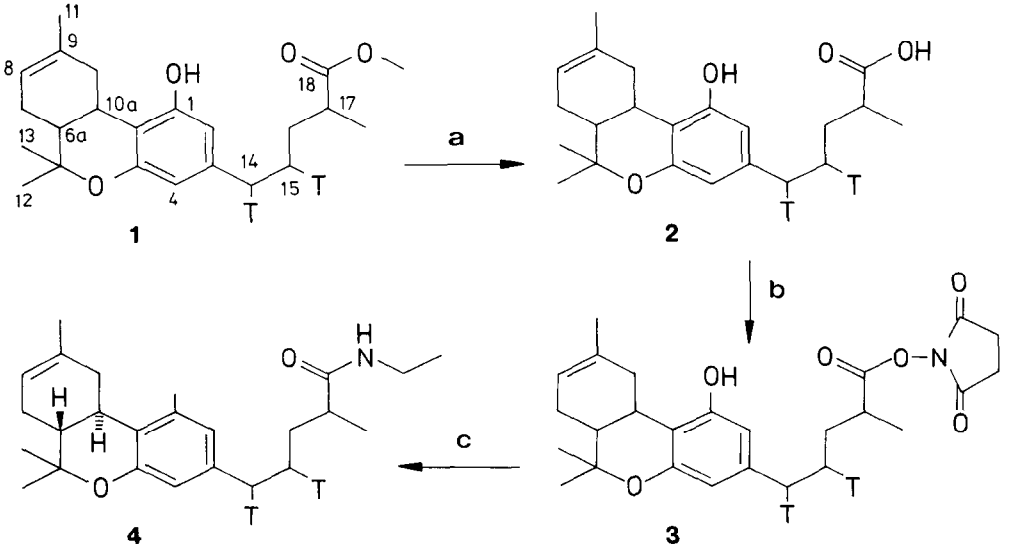


Fig. 1. Section of the basic structure of the affinity gel.

yield was reacted with *N*-hydroxysuccinimide in anhydrous acetonitrile in the presence of dicyclohexylcarbodiimide. The resulting *N*-hydroxysuccinimide ester **3** was transformed into amide **4** in 70% yield using ethylamine in H_2O/dioxane. Chromatography on silica gel gave pure amide **4** (specific activity 7.27 mCi/mmole); for analytical data see: [6]. The compound was stored in ethanol in the dark at −20°.

2. *Pharmacokinetics.* Male Han:NMRI mice (18–22 g) were obtained from the Zentralinstitut für Versuchstiere, Hannover. Stock solutions of amide **4** were prepared for i.v. injection in polyethylene glycol/H_2O 4:1 (v/v) (2.34 mmoles/l **4**) and for i.p. injection in physiological saline containing 10% (v/v) propane-1,2-diol and 1% (w/v) Tween-80 (4.68 mmoles/l **4**). Injection vol. for i.v. (tail vein) and i.p. pharmacokinetics: 0.1 ml per mouse, injected amounts of amide **4**: i.v. 90 μg ≙ 234 nmoles ≙ 3.75×10^6 dpm; i.p. 180 μg ≙ 468 nmoles ≙ 7.5×10^6 dpm. Animals were killed by cervical fracture 5, 15, 30, 45, 60 and 120 min after i.v. injection and 7.5, 15, 30, 45, 60, 90 and 120 min after i.p. injection and perfused with 30 ml 0.9% NaCl solution. Total brains were removed, rinsed with NaCl solution, dried on filter paper and weighed.



Scheme 1. Synthesis of amide **4**. Reaction conditions: (a) KOH in CH_3OH, 30 min reflux; (b) *N*-hydroxy-succinimide in abs. CH_3CN, dicyclohexylcarbodiimide, 24 hr, 23°; (c) $NH_2C_2H_5$, $NaHCO_3$ in H_2O/dioxane, 90 min, 23°.

After longitudinal dissection half of each brain was digested in 2.0 ml tissue solubilizer (TS-1, Zinsser) at 50° for 20 hr and bleached by addition of 0.4 ml 2-propanol and 0.4 ml 30% H_2O_2 at 40° for 30 min. Radioactivity was assayed by counting samples of 1.0 ml in 10 ml Unisolve 1 (Zinsser). Chemoluminescence was suppressed by adding 50 μl acetic acid to each sample.

3. *Triglyceride/phospholipid partitioning.* This method, originally described in [7] and applied to cannabinoids by us [5] has been described in detail. In addition to the reported system consisting of egg phosphatidylcholine and glycerol trioleate a defined system using oleoyl palmitoyl phosphatidylcholine was employed. Amide **4** was added in total amounts of 1.2 and 5.0 nmoles to both systems and subjected to the partitioning procedure. After equilibrating the partitioning mixture, followed by centrifugation, each centrifuge tube was frozen and cut into five slices. Radioactivity of each slice was assayed by liquid scintillation counting.

4. *Test for cannabis-like activity.* Drug discrimination is a reliable way to test a compound for cannabis-like activity in animals. The system originally described in [8] was established in our laboratory and has been discussed in detail [9]. Eleven male albino rats (WISTAR, Zentralinstitut für Versuchstiere, Hannover, 350–430 g) were trained to discriminate between 3 mg/kg Δ^9-THC and its vehicle in a two-choice task represented by a T-shaped water maze. Δ^9-THC and amide **4** were emulsified in physiological saline containing 10% (v/v) propane-1,2-diol and 1% (w/v) Tween-80 in physiological saline at concentrations up to 12 mg/ml. Twenty mg/ml of amide **4** required 18% Tween-80. The drugs were injected i.p. in a constant vol. of 1 ml/kg. The injection/trial interval was kept at 40 min.

RESULTS

1. *Pharmacokinetics*

Results are expressed in total brain radioactivity in % of the injected dose. Each value is averaged from six animals and corrections were made to account for differences in brain wt (cf. [5]).

Figure 2 gives the total brain cannabinoid concentrations (inclusive potential metabolites) and the lowest and highest value of each group after i.p. and i.v. injection of the amide. The graph includes the previously published i.v. data [5] of Δ^9-THC and methyl 17(*R,S*)-methyl-(6a*R*,10a*R*)-Δ^8-THC-18-oate **1**, the ester analogue of amide **4** to facilitate the subsequent discussion.

Given intraperitoneally, the amide reaches peak concentrations between 0.11 and 0.16% at 15 min followed by a slow decrease to 0.05–0.08% at 120 min. Upon i.v. injection, the amide is taken up readily into the brain, peak concentrations of 1.9% (average) being attained at about 5 min. This high initial accumulation is followed by a rapid biphasic drop in brain radioactivity. The initial and terminal excretion half lives of the amide were calculated as $T_{1/2}$(INIT) = 12.5 min and $T_{1/2}$(TERM) = 60 min. At 120 min brain concentrations of 0.1% are reached. Especially after i.v. injection the pharmacokinetic properties of the amide are markedly different from those of Δ^9-THC and the ester.

2. *Triglyceride/phospholipid partitioning*

During the centrifugation of the partitioning mixture the triglyceride accumulates at the top of the centrifuge tube, the phospholipid at the bottom corresponding to slices 1 and 5 respectively. Taking into account the mutual solubility of the lipids, the partitioning coefficients of cannabinoids are expressed as mole fractions $K_{\text{glycerol}tri\text{oleate/Phosphat-}}$

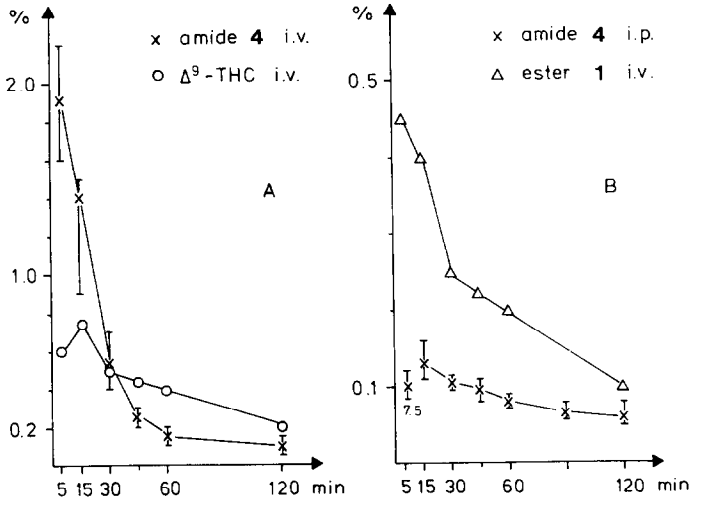


Fig. 2. Brain levels of the amide **4** (in % of total radioactivity) in mice after i.v. (A) and i.p. (B) injection (×). All values are averaged from six animals. The highest and lowest values of each group are indicated. The corresponding data of Δ^9-THC (○) and ester **1** (△) [5] are included in A and B resp.

idylcholine ($K_{TRI/PC}$). Using egg phosphatidylcholine as phospholipid a $K_{TRI/PC}$ of 0.063 was obtained for amide **4**, with oleoyl palmitoyl phosphatidylcholine the $K_{TRI/PC}$ was 0.127. The $K_{TRI/PC}$ values for Δ^9-THC and the ester, as determined in the less defined system, were 0.88 and 0.40 respectively (cf. [5]).

Again, the data for Δ^9-THC and the ester are included for comparison. Both amide **4** and the ester are mostly found in the phospholipid, i.e. exhibit higher affinity for cell membranes than Δ^9-THC.

3. *Cannabis-like activity*

When tested in our rats, amide **4** was generalized to the training drug Δ^9-THC (3 mg/kg). At 5 mg/kg amide one animal out of 11 (1/11), at 7.5 mg/kg (4/11), at 10 mg/kg (7/11) and at 20 mg (11/11), gave drug responses. From these data the ED_{50} of the amide (i.e. the dosage leading to 50% drug responses) was calculated as described [9]. Plotting the drug response probability P(x) vs dosage x in log scale the fitted curve shown in Fig. 3 was calculated revealing an ED_{50} value of 8.7 mg/kg for the amide **4**. Compared to the training drug Δ^9-THC with an ED_{50} of 1.8 mg/kg, the amide is about 5 times less potent.

DISCUSSION

The aim of the studies presented here was to investigate whether 17(R,S)-methyl-(6aR,10aR)-Δ^8-THC-18-oic-acid **2** is a suitable ligand for the affinity chromatography approach to isolate the THC receptor. This was achieved by the evaluation of the properties of amide **4** which represents the closest chemical approximation to the final affinity unit. The amide readily crosses the blood–brain barrier. In order to function as affinity ligand, this condition must not be met but it is a prerequisite for cannabis-like activity. After i.v. injection the amide reaches brain concentrations 5 and 3 times higher than the peak values observed with the ester and Δ^9-THC resp. This large difference, especially in comparison to the chemically closely related ester and the biphasic excretion half lives indicate the involvement of a transport system for the amide. With Δ^9-THC only a single $T_{1/2}$ (57 min) is observed, comparable to the $T_{1/2}$(TERM) (60 min) of the amide. Upon i.p. injection the amide's pharmacodynamic properties are less spectacular. Peak concentrations of 0.13% are reached within 15 min followed by a slow decline of brain radioactivity.

Injected intravenously, both amide and ester show maximal brain levels after 5 min, Δ^9-THC after 15 min. This may be explained by the results of the triglyceride/phospholipid partitioning. Δ^9-THC distributes almost equally between triglyceride (i.e. adipose tissue) and phospholipid (i.e. membranes) phases ($K_{TRI/PC} = 0.88$). Since the brain virtually contains no triglycerides, the slow increase in brain concentration of Δ^9-THC is due to redistribution phenomena. The amide ($K_{TRI/PC} = 0.063$) and the ester ($K_{TRI/PC} = 0.40$) exhibit a marked preference for the phospholipid phase and no redistribution takes place. Finally the discriminative stimulus properties of the amide were generalized to Δ^9-THC in rats. This proves the cannabis-like activity of the amide and indicates that the novel model compound retains affinity to the postulated receptor. If the pharmacokinetic data obtained in mice after i.v. injection may be transferred to the drug discrimination experiment, at 40 min, the time at which the tests are run, about twice as much Δ^9-THC than amide would be present in the brain of the rats. Compared to the relative potency obtained from the ED_{50} values of the amide and Δ^9-THC, this doubles the cannabis-like potency of the amide at the receptor level making it only 2.5 times less potent than Δ^9-THC itself.

Thus, the data reported here justify our choice of 17(R,S)-methyl-Δ^8-THC-18-oic acid as cannabinoid affinity ligand.

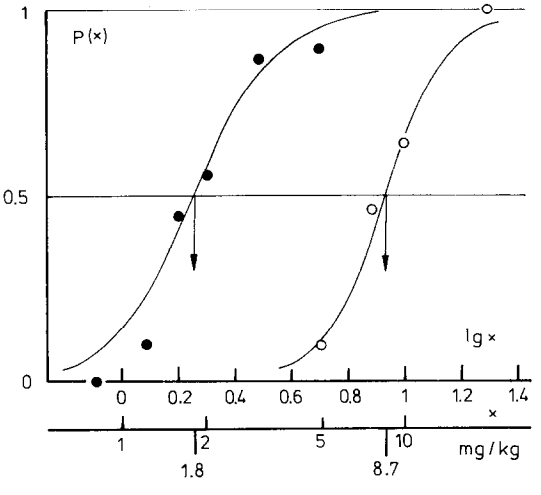


Fig. 3. Dose/response curves for the amide (○) and Δ^9-THC (●) (data for Δ^9-THC from [9]). Response probability P(x) is plotted vs dosage x in log scale. The fitted curves are given by the equations: for the amide, $P(x) = 1/1 + \exp -(-8.39 + 8.95x)$, for Δ^9-THC, $P(x) = 1/1 + \exp -(-1.83 + 7.14x)$.

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