Determination of Δ^9-tetrahydrocannabinol levels in brain tissue using high-performance liquid chromatography with electrochemical detection

Emson C. Nyoni[a,*], Balvant R. Sitaram[a], David A. Taylor[b]

[a]*Department of Pharmaceutics, Victorian College of Pharmacy, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia*
[b]*Department of Pharmaceutical Biology and Pharmacology, Victorian College of Pharmacy, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Victoria 3052, Australia*

Abstract

Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the major psychoactive component of cannabis. To assist in investigating the mechanism(s) of action of Δ^9-THC, a convenient method for determining its levels in brain tissue is required. We now describe a method for determining nanogram quantities of Δ^9-THC in rat brain tissue. The method employs solvent extraction with methanol–hexane–ethyl acetate, followed by analysis using liquid chromatography with electrochemical detection. Overall recoveries were greater than 80%. The relationship between the peak-height ratio for processed standards extracted in the presence of tissue (Δ^9-THC/internal standard) and the amount of Δ^9-THC added was shown to be linear within the range of concentrations examined. Quantitative measurements of Δ^9-THC in different brain regions following the intravenous administration of Δ^9-THC are presented as examples of the applications of this method.

1. Introduction

A number of studies have reported significant effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive component of cannabis, on the level of the monoamine neurotransmitters, 5-hydroxytryptamine and dopamine, and their metabolites in brain tissue [1–4]. In recent studies [5,6] it has been demonstrated using microdialysis techniques that the intravenous administration of Δ^9-THC may effect the neuronal release of dopamine (as measured

by changing extracellular concentrations) in the corpus striatum. To examine in detail the relationship between the changes in the release of monoamine neurotransmitters and concentration of Δ^9-THC present in discrete brain regions, such as the corpus striatum, a sensitive and selective method for the analysis of Δ^9-THC in discrete regions of the brain is required.

Although a number of gas chromatographic (GC) [7–10], gas chromatographic–mass spectrometric (GC–MS) [11] and radioimmunoassay (RIA) methods [12] for the analysis of Δ^9-THC in biological fluids such as blood, urine and saliva have been

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*Corresponding author.

described, few detailed procedures for the specific application of these techniques to the analysis of Δ^9-THC in brain tissue have been outlined. While GC with electron-capture detection and GC–MS methods potentially meet our sensitivity requirements, elaborate sample preparation procedures and/ or the need for derivatisation prior to analysis place limitations on their utility. Available RIA assays meanwhile, while sufficiently sensitive, appear to lack the necessary selectivity.

Among the more recent developments, liquid chromatography (LC) combined with ultraviolet absorption or electrochemical detection (LC–ED) has formed the basis for improved methods for the analysis of Δ^9-THC in biological matrices [13–17]. While a number of reports have outlined the use of sensitive LC–ED techniques for the analysis of Δ^9-THC and its metabolites in plasma, urine and saliva [15–17], the specific use of similar techniques for the analysis of Δ^9-THC in brain tissue has not yet been described. A number of drawbacks attributed to inadequate sample preparation, poor liquid chromatographic separation and the lack of an appropriate internal standard also appeared likely to limit the selectivity and reliability of available methods.

We now describe a method for determining nanogram amounts of Δ^9-THC in discrete regions of the brain, and illustrate the use of this method for the determination of the Δ^9-THC levels in brain following intravenous administration, with particular reference to the corpus striatum.

2. Experimental

2.1. Materials

Δ^9-THC was supplied by the National Institute on Drug Abuse (Rockville, MD, USA). 4-Dodecylresorcinol was obtained from Aldrich (Milwaukee, WI, USA) and polyvinylpyrrolidone (PVP; M_r 30 000) was purchased from BASF (Melbourne, Australia). All solvents used were of analytical or LC standard. Water was glass-distilled prior to use.

2.2. Animals

Female Glaxo Wistar rats, supplied by the Victorian College of Pharmacy Animal House, were maintained in a temperature-controlled environment (22°C) and subjected to a 12h:12h light–dark cycle. Food and water were available to animals ad libitum.

2.3. Instrumentation

Chromatography was performed using a Waters 510 liquid chromatograph (Millipore Waters, Milford, MA, USA). All samples were introduced into the column by means of a Rheodyne 7125 injector fitted with a 100-μl loop. Detection was achieved using a Bioanalytical Systems LC-4B dual-electrode electrochemical detector, fitted with a TL-5A glassy carbon working electrode (Bioanalytical Systems, West Lafayette, IN, USA), operated at 1.2 V against a Ag/AgCl reference electrode. Outputs were recorded on a BBC Goerz Metrawatt SE 120 dual-channel chart recorder.

2.4. LC

Separation of Δ^9-THC was achieved by reversed-phase LC on an ODS column (Waters μBondapak C_{18}, 300 × 4 mm I.D., 10 μm) protected by an RP-18 Newguard pre-column cartridge (Brownlee, Santa Clara, CA, USA). The mobile phase was of methanol–acetonitrile–0.01 M H_2SO_4 (21:24:55, v/v) and was maintained at a flow-rate of 3.0 ml/ min. The column temperature was maintained at 22°C, using a Waters TCM column oven. To ensure base-line stability, the mobile phase was continuously recycled.

2.5. Preparation of Δ^9-THC for injection

Δ^9-THC for injection was prepared according to the method of Fenimore and Loy [18]. To Δ^9-THC (100 mg) in 1 ml of ethanol was added 2 g of the suspending agent, PVP. After careful mixing, the solvent was evaporated to complete dryness at 30°C under vacuum, using a Bucchi Rotary Evaporator. The residue was suspended in isotonic saline to give a final Δ^9-THC concentration of 5 mg/ml.

2.6. Procedure for the extraction of Δ^9-THC from brain tissue

Following the intravenous administration of Δ^9-THC via an in-dwelling jugular catheter, animals

were killed by cervical dislocation and the brain rapidly removed and placed at 0°C. Regions of the brain to be examined were then dissected.

Accurately weighed samples of brain tissue (approximately 50 mg) were placed in 16-ml polypropylene Sorvall centrifuge tubes and 2.5 ml of methanol containing 700 ng of the internal standard, 4-dodecylresorcinol, were added. The tissue was initially homogenised in the centrifuge tubes using a closely fitting 13 mm O.D. Teflon pestle (Thomas Scientific, Swedesboro, NJ, USA). The tissue homogenates were then sonicated using a Soniprep 150 ultrasonic disintegrator (MSE Scientific Instruments, Sussex, UK), fitted with a probe assembly with a 3.0-mm diameter tip. The probe was operated at an amplitude of 23 μm. Tissue homogenates were maintained in an ice-bath and subjected to three 2-min intervals of sonication.

After disruption of the tissue, the homogenates were centrifuged at 15 000 g in a Model RC-2B Sorvall high-speed centrifuge (Dupont, Newtown, CT, USA) for 30 min at 4°C. The supernatant was then transferred to a 10-ml conical glass centrifuge tube and evaporated to dryness in a Model RC101 Jouan centrifugal Evaporator (Jouan, Saint-Herblain, France). The residue was reconstituted in 5 ml of hexane–ethyl acetate (7:3, v/v) and the organic phase was washed with three 2.5-ml aliquots of 0.05 M H_2SO_4. After centrifugation at 3000 g at 4°C in a Sorvall AT6000B refrigerated centrifuge, the aqueous phase was withdrawn and discarded. Following the final wash, a 4.0-ml aliquot of the organic phase was transferred to a 10-ml conical glass centrifuge tube and evaporated to dryness using the centrifugal evaporator. The residue was reconstituted in a 350-μl aliquot of mobile phase–methanol (25:10, v/v). A 50-μl sample was then subjected to liquid chromatographic analysis.

2.7. Preparation of standard extracts

Samples of cortex (50 mg) obtained from untreated animals were placed in polypropylene Sorvall centrifuge tubes and 2.15 ml of methanol were added. Aliquots (350 μl) of standard solutions containing internal standard (4-dodecylresorcinol, 1 μg/ml) and concentrations of Δ^9-THC in the range 0–1.0 μg/ml were then added. The tissue was homogenised and the Δ^9-THC extracted as described

in Section 2.6. Overall recoveries (%) were determined by a comparison of the peak heights obtained to those of appropriate standards.

3. Results

3.1. LC

Reversed-phase LC performed on a μBondapak C_{18} column permitted the complete separation of Δ^9-THC and the proposed internal standard, 4-dodecylresorcinol, with an overall analysis time of less than 15 min (Fig. 1b). To achieve optimum sensitivity (maximum signal to noise) electrochemical detection was performed at 1.2 V. Attempts to use higher voltages resulted in unacceptable noise and baseline drift.

3.2. Validation of the analysis

On each day, repeated analyses of 50-μl aliquots of standard solutions containing 0.04, 0.1, 0.6 and 1.0 μg/ml of Δ^9-THC (equivalent to 2, 5, 30 and 50 ng of Δ^9-THC on-column, respectively) in methanol were performed by reversed-phase LC and yielded coefficients of variation for the determinations of less than 2% ($n=5$; $p<0.05$). Linear relationships were observed between the detector response (peak-height ratio of standard/internal standard) and the concentration of Δ^9-THC, following chromatography on the μBondapak C_{18} column, within the range of concentrations examined (0–10 μg/ml).

The minimum detectable limit for Δ^9-THC under the conditions described (determined two days after polishing of the glassy carbon electrode) was 1.5 ng on-column (signal-to-noise ratio of 2:1).

3.3. Extraction of Δ^9-THC from brain tissue

Protein precipitation and the initial extraction of Δ^9-THC from the brain tissue of animals that had been given Δ^9-THC intravenously, was attained by homogenising samples of tissue in methanol. Following removal of the methanol by evaporation, further purification of the sample was achieved by solvent extraction using a mixture of hexane–ethyl acetate. Additional impurities were removed by repeated
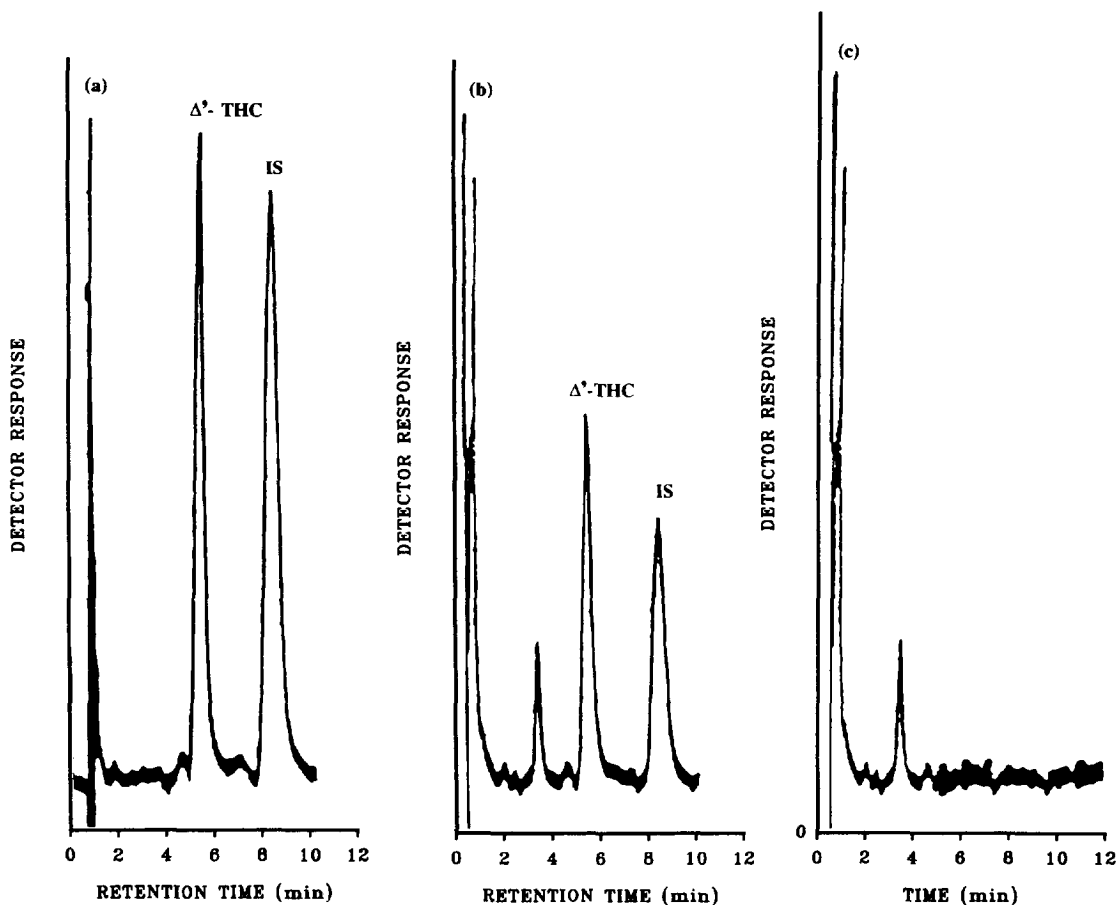
Fig. 1. Chromatograms obtained following the analysis of a 50-μl aliquot of (a) a standard solution containing Δ^9-THC (0.1 μg/ml) and the internal standard 4-dodecylresorcinol (I.S.) and (b) an extract prepared from the corpus striatum of a rat injected with 2 mg/kg of Δ^9-THC and killed 10 min later. Chromatogram (c), prepared from a vehicle-treated control without the addition of the internal standard, is also presented. Standard solutions and brain tissue extracts were prepared and analysed as described in Section 2.

washing of the organic phase with 0.05 M H$_2$SO$_4$. After evaporation of the organic phase, the residue containing the extracted Δ^9-THC was finally reconstituted in a mixture containing mobile phase and methanol. The composition of the reconstitution medium was selected to avoid any baseline perturbations at the elution times for Δ^9-THC and the internal standard.

A chromatogram obtained following the analysis of an extract prepared from the rat corpus striatum 10 min after the administration of 2 mg/kg Δ^9-THC is presented in Fig. 1b and illustrates the separation of Δ^9-THC and the internal standard from other constituents in the brain tissue extract. The analysis of a similar extract from the cortex of a vehicle-treated control (Fig. 1c) confirmed the absence of any co-eluting compounds which might interfere with the determination of either Δ^9-THC or the internal standard used.

3.4. Quantitation

Brain tissue samples (cortex) from untreated animals (50 mg) spiked with internal standard (350 ng) and amounts of Δ^9-THC within the range 0–350 ng, were extracted and analysed using reversed-phase

LC as described in Section 3.1. These were compared to non-extracted standards. The overall recoveries of Δ^9-THC from brain tissue using the method described was 80% with C.V. values of less than 5% ($n=5$; $p<0.05$). The relationship between the peak-height ratio for processed standards extracted in the presence of tissue (Δ^9-THC/internal standard) and the amount of Δ^9-THC added was shown to be linear within the range of concentrations examined.

The peak-height ratios determined for standards extracted in the presence of tissue were lower than those obtained in the absence of tissue (data not shown). Consequently, all quantitations of brain tissue levels of Δ^9-THC following drug administration were routinely conducted with reference to standards processed in the presence of tissue. Processed standards were run on a daily basis.

3.5. Application of the LC analysis method to the study of Δ^9-THC pharmacokinetics in rat brain

The methods for the extraction and LC analysis of Δ^9-THC described in Section 2.6 and Section 3.1 were developed to permit the determination of Δ^9-THC levels in rat brain after the administration of pharmacologically relevant doses of Δ^9-THC. Using the methods described, both time-dependent (Fig. 2a) and dose-dependent (Fig. 2b) increases in the concentration of Δ^9-THC in the cortex and in the corpus striatum could be clearly discerned, after the intravenous administration of a 2 mg/kg dose of Δ^9-THC. No statistically significant regional differences in the distribution of the drug in these two brain areas was evident. At doses of THC below 5 mg/kg i.v., the level of THC determined in the brain regions appeared to be linearly related to the dose administered. At a dose of 10 mg/kg of THC, this apparent linear relationship did not hold (Fig. 2b).

4. Discussion

A major goal in marijuana research is the elucidation of the mechanism of action of the psychoactive compound Δ^9-THC. The characterisation of the effects of Δ^9-THC on the central nervous system of animals forms an important component of this research. To facilitate such studies, we have developed methods for the determination of the levels of Δ^9-THC in rat brain, following drug administration. The procedures described permit the quantitative analysis of nanogram quantities of Δ^9-THC in discrete brain regions. The extraction procedures that we have described involve the disruption of brain tissue in methanol, a water-miscible solvent in which Δ^9-THC is known to have a high solubility. A combination of homogenisation and sonication was used to optimise the overall recovery of Δ^9-THC from brain tissue. Subsequent sample purification from other brain constituents, to minimise interference with the determination of Δ^9-THC levels, was achieved by solvent extraction.

This method is suitable for the study of the bioavailability and pharmacokinetics of Δ^9-THC in discrete brain regions. Using this method, we were able to study the bioavailability and phar-
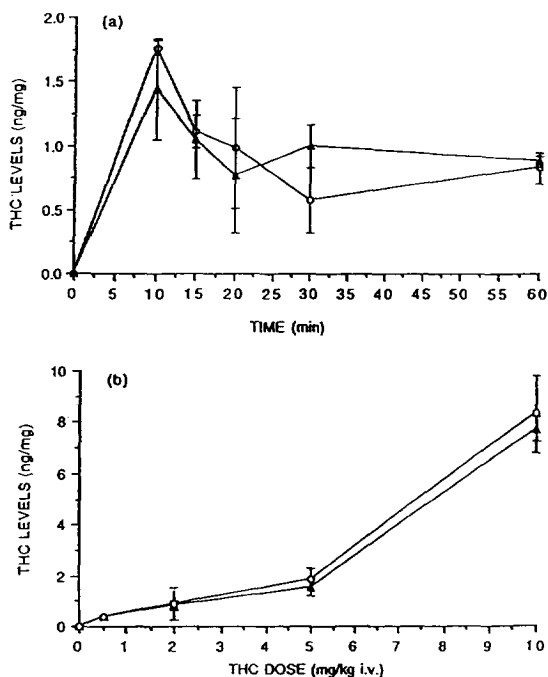

Fig. 2. The level of Δ^9-THC present (a) in the corpus striatum (▲) and cortex (○) of rats killed at various intervals following the intravenous administration of Δ^9-THC (2mg/kg) and (b) in rats killed 10 min following the intravenous administration of various doses of Δ^9-THC. Brain tissue extracts were prepared and analysed as described in Section 2. Results are presented as the mean ± S.D. of determinations carried out on five animals.

macokinetics of Δ^9-THC in discrete brain regions. Given the similarity of our chromatographic conditions to the method of Nakahara et al. [15], it is possible that other cannabinoids may be similarly determined in brain tissue using this method.

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