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Distribution of Δ^9-tetrahydrocannabinol in the mouse

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Summary

1. The distribution of Δ^9-tetrahydrocannabinol-^{14}C in the pregnant and non-pregnant mouse is very similar. High concentrations of radiolabel can be seen in the maternal liver, spleen, lungs, brown fat, adrenal glands, mammary glands, yolk sac placenta and corpora lutea. In the pregnant mouse Δ^9-THC crosses the placenta and enters the foetuses in very low concentrations, with no apparent selective intrafoetal radiolabel accumulation sites. Autoradiograms showing the distribution of ^{14}C-cannabinoid 2 h after dosing are presented.

2. A small amount of maternal liver and foetal tissue was removed from the mice, used for autoradiography and extracted with ethyl acetate. Most of the radiolabel in these tissues was solvent extractable and was separated by thin layer chromatography into two fractions, THC and metabolites.

3. It appears that most of the cannabinoid is present in a free rather than conjugated form.

Introduction

Several studies on the chemical constituents of marihuana (*Cannabis sativa* L.) have been reported in the literature (Garattini, 1965; Hively, Mosher & Hoffman, 1966). Δ^9-Tetrahydrocannabinol (Δ^9-THC) is the major psychotomimetically active constituent of cannabis (Hollister, Richards & Gillespie, 1968; Mechoulam, 1970). More recently, the structure and biological activity of two metabolites of Δ^9-THC has been reported by this laboratory (Wall, Brine, Brine, Pitt, Freudenthal & Christensen, 1970; Christensen, Freudenthal, Gidley, Rosenfeld, Boegli, Testino, Brine, Pitt & Wall, 1971) where it was shown that the 11-hydroxy metabolite of Δ^9-THC is considerably more potent than the parent compound when administered intracerebrally. Although the metabolism of Δ^9-THC in the mouse has been reported, very little is known about the distribution of this agent in the intact animal.

In the present investigation the distribution of Δ^9-THC in both pregnant and non-pregnant mice has been studied using the Ullberg method of whole body autoradiography (Waddell & Brinkhous, 1967). This has permitted the simultaneous comparison of the concentrations of radiolabel in the maternal tissues, the placenta and the foetal tissues at specific times after drug administration. It addition, specific tissues were removed from the portion of frozen mouse remaining after sufficient slices were taken for autoradiography, and the radiolabel from these tissues was extracted to determine the actual amount of cannabinoid remaining in the form of Δ^9-THC at the time of sacrifice and whether it is in a free or conjugated form.

Methods

Carworth CF-1 female mice were used. Brinkmann precoated silica gel F-254 thin layer chromatography plates (tlc) were used for the separation of cannabinoids.

The ^{14}C-Δ^9-tetrahydrocannabinol (THC) (specific activity: 17·5 μCi/mg) was synthesized by Dr. Colin Pitt of this laboratory. Four pregnant mice were used on gestation day 15. The drug was administered in 0·1 ml ethanol. At 30 min and 2 h after the oral administration of 0·65 mg (11·5 μC) Δ^9-THC, two pregnant and two non-pregnant mice at each time period were anaesthetized with diethylether and immediately immersed into a dry ice-hexane bath (−78° C). Since a detailed description of the methodology is available in the literature (Waddel & Brinkhous, 1967) only a brief description will be presented. The frozen mouse is placed on its side in a mould containing a concentrated solution of carboxymethylcellulose (CMC). The bottom of this mould is the stage for the model 1300 Leitz microtome. The mould containing the precooled CMC solution and frozen mouse is lowered into a dry ice-hexane bath for approximately 3 min after which the sides of the mould are removed and the CMC block is mounted on the microtome located in a chest type freezer. Sagittal sections 40 μm thick are cut through the mouse and mounted on Scotch cellophane tape, dehydrated and then exposed by apposition against X-ray film. After an appropriate exposure time (for this study approximately 9 days) the film is developed thus producing an autoradiogram.

The small amount of foetal tissue and maternal liver in the remaining unsectioned quarter of the mouse was carefully removed and separately extracted 5 times with redistilled ethyl acetate. The radiolabelled cannabinoids thus removed were spotted on tlc plates and developed in a solvent system containing acetone and chloroform (1:4). A reference standard for Δ^9-THC was also spotted on the plate apart from the extracted material. The developed tlc plates were scanned on a Packard Model 7201 radiochromatogram scanner and compared with the developed reference compound. The areas on the tlc plate corresponding to the drug and metabolites were scraped into scintillation vials and counted in a Packard Tri-Carb Scintillation Spectrometer to determine the percentage of each component present in the tissue at the time of sacrifice.

A counting sample of the remaining aqueous fraction indicated the presence of a significant number of counts which could not be extracted with ethyl acetate. These radiolabelled compounds could be either polar metabolites, which are preferentially soluble in the aqueous phase or conjugates of cannabinoids which when unconjugated are readily extractable in an organic solvent. To determine the nature of the radiolabelled material, the aqueous phase from each tissue was incubated with 1,000 units of sulphatase and β-glucuronidase from *Helix pomatia*, after which the aqueous phase was again extracted with redistilled ethyl acetate. The extracted radiolabel was counted as previously described.

Results

The experiment described in **Methods** was performed twice. In the first experiment, Δ^9-THC was administered intravenously (i.v.) to the eight mice. The autoradiograms from mice 30 min and 2 h after injection were essentially identical, except that the 2 h autoradiograms showed considerably higher concentrations of radiolabelled material in the specific sites of drug localization. Figure 1a is an autoradiogram of a pregnant mouse sacrificed 2 h after Δ^9-THC administration. In Fig. 1a, accumulation of radiolabel can be seen in the following maternal tissue: liver, spleen, lungs, brown fat, adrenal gland, mammary glands and the yolk sac placenta. The foetuses show only a very low level of radiolabel with no selective

intrafoetal radiolabel accumulation sites. The non-pregnant mice to which Δ^9-THC was administered intravenously before sacrifice showed a distribution pattern identical to that of the pregnant mice. A detailed list of the tissue localization of high radioactivity in both pregnant and non-pregnant mice is presented in Table 1.

Because Δ^9-THC is very lipid soluble and almost insoluble in aqueous media, the small circular black spots in the lungs and spleen (Fig. 1a) corresponding to areas of very high radiolabel accumulation could be either true sites of high radiolabel localization or sites of accumulation of Δ^9-THC microdroplets resulting from the failure of the cannabinoid to remain in solution after intravenous administration. Should these sites of high radiolabel concentration be true receptor sites, then a very similar pattern in both the lungs and spleen should be seen when the drug is administered orally. To determine the reason for this accumulation (receptor sites or insolubility), the experiment was repeated changing only the route of drug administration from intravenous to oral, using an intubation needle to place the drug directly into the stomach.

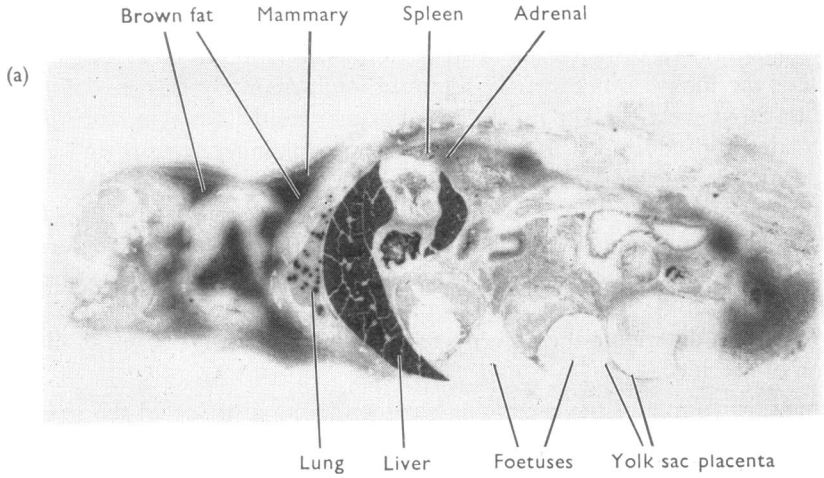

FIG. 1a. An autoradiogram from a 40 μm sagittal mouse section showing the distribution of Δ^9-THC (11·5 μCi-C^{14}) in a 15-day pregnant mouse 2 h after intravenous injection. Dark areas correspond to high concentration of radioactivity. Radiolabel accumulation can be seen in the maternal liver, spleen, lung, brown fat, mammary glands, adrenal gland and yolk sac placenta.

TABLE 1. *Mouse tissues showing relatively high radiolabel localization*

Tissue	Dosed intravenously	Dosed orally
Adrenal (cortex)	++	++
Brown fat	++	++
Corpora lutea	++	++
Foetus	0	0
Harders gland	++	++
Intestine	+	++
Kidney	+	+
Liver	++	++
Lungs	++	+
Mammary glands	+	+
Myocardium	0	+
Spleen	++	+
Stomach	+	+
Urinary bladder	+	+
Yolk sac placenta	+	+

++, Very high radiolabel localization; +, radiolabel significantly higher than remaining tissues.

Figure 1b is an autoradiogram of a pregnant mouse sacrificed 2 h after the oral administration of Δ^9-THC. High concentrations of radiolabel can be seen in the liver, intestine, brown fat, yolk sac placenta, kidney and mammary glands. The concentration of radiolabel in the lungs (Fig. 1b) is higher than that in the general mouse musculature but significantly lower and more evenly dispersed than that found both in the other tissues listed above and in the lungs shown in Fig. 1a. No specific sites of localization could be found in either the lungs or spleen in the mice given Δ^9-THC orally.

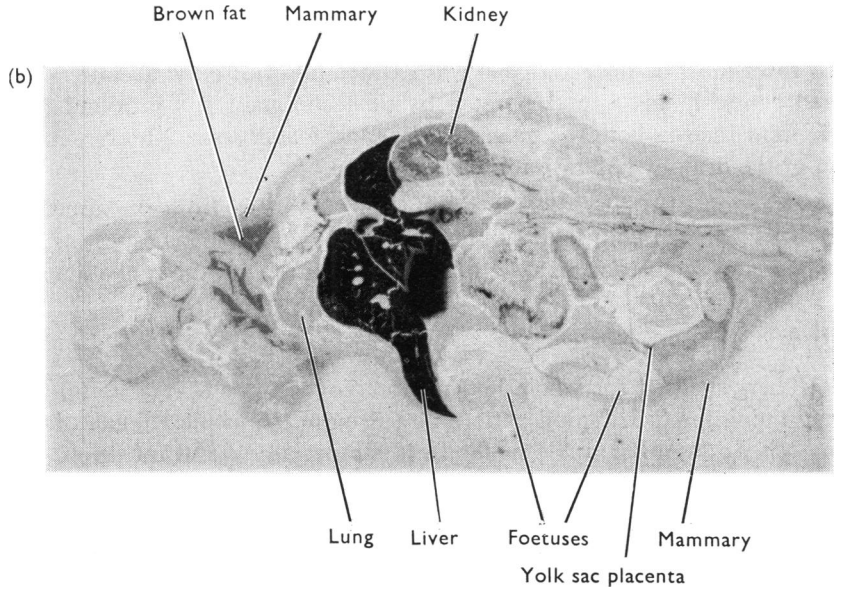

FIG. 1b. An autoradiogram showing the distribution pattern of Δ^9-THC (11·5 μCi-C^{14}) in a 15-day pregnant mouse 2 h after oral administration. High radiolabel concentration can be seen in the liver, intestine, and brown fat, and to a lesser extent in the mammary glands, kidney, and yolk sac placenta.

TABLE 2. *Radioactivity in tissue removed 2 h after administration*

		Fraction	Pregnant mouse (i.v.) Liver	Pregnant mouse (i.v.) Foetus	Pregnant mouse (oral) Liver	Pregnant mouse (oral) Foetus	Non-pregnant (i.v.) Liver
I	(a)	% of total radiolabel extracted from the aqueous with EtOAc	80·3	68·2	68·2	93·2	73·9
	(b)	TLC separation of the EtOAc extract*					
		1. Δ^9-THC	21·8	16·6	8·0	13·9	32·2
		2. Metabolites	45·7	38·4	48·2	73·6	31·6
II	(a)	% of total radiolabel remaining in the aqueous fraction	19·7	31·8	31·8	6·8	26·1
III	(a)	% of EtOAc extractable radiolabel after enzyme hydrolysis of the aqueous†	60·0	39·7	31·8	60·0	16·9
		1. Δ^9-THC	10·9	18·8	3·2	17·8	0·2
		2. Metabolites	89·1	81·2	96·8	82·2	99·8
	(b)	% of radiolabel remaining in the aqueous phase†	40·0	60·3	68·2	40·0	83·1

* Expressed as a percentage of the total radioactivity (EtOAc extractable and aqueous). † Expressed as a percentage of IIa.

Figure 1c shows the very high concentration of radioactivity in the corpora lutea (found in all eight pregnant mice), in addition to that in the yolk sac placenta and mammary glands.

The results from the ethyl acetate extraction of the maternal livers and foetal material are presented in Table 2. As can be seen from the autoradiograms, the maternal liver contained a much higher concentration of radiolabel than the foetuses. However, enough liver and foetal tissue was recovered from the mouse section to provide a radioactivity recovery range from 21,470 d.p.m. to 191,100 d.p.m. A representative set of data obtained from one mouse from each set representing orally dosed pregnant, intravenously dosed pregnant and non-pregnant mice is presented in Table 2.

In all cases most of the radiolabel was extractable with ethyl acetate. When the extracted cannabinoids were separated by tlc techniques, it was found that some $\triangle^9$-THC remained in both the maternal liver and foetal tissue 2 h after the administration of the drug by either route.

The extraction of a considerable amount of radiolabel from the aqueous phase after it was incubated with both sulphatases and β-glucuronidase suggests that both the mothers and foetuses were able to form conjugates with the cannabinoids.

Discussion

The Ullberg method for whole body autoradiography is a very useful technique for determining the distribution pattern of a drug in the whole animal. In contrast to many other autoradiographic methods, it is especially useful for drugs which are

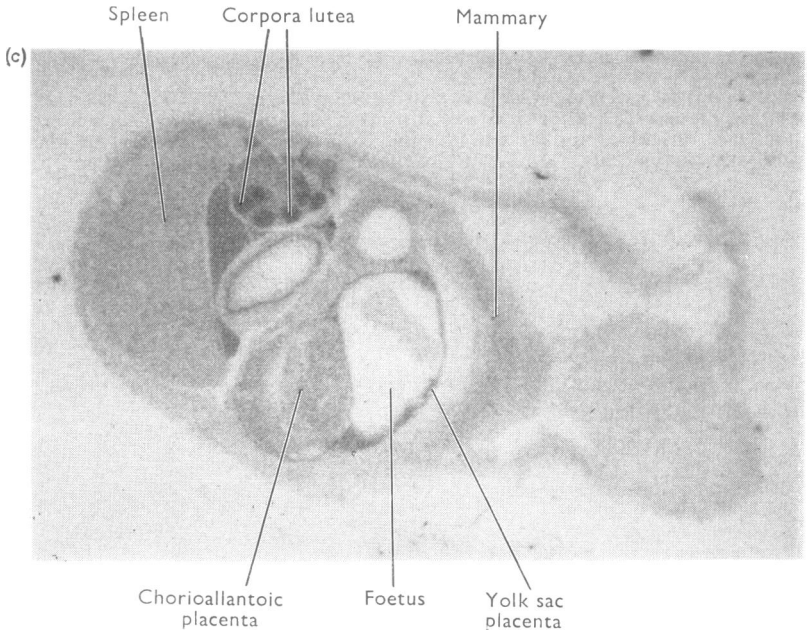

FIG. 1c. An autoradiogram showing the distribution pattern of $\triangle^9$-THC (11·5 μCi-C^{14}) in a 15-day pregnant mouse 2 h after oral administration. High radiolabel concentration is seen in the corpora lutea and yolk sac placenta with slightly less in the chorioallantoic placenta and mammary gland.

very soluble in organic solvents (that is $\triangle^9$-THC) for the tissues never come in contact with any solvent that might cause diffusion of the isotope.

The general distribution pattern of ^{14}C-labelled $\triangle^9$-THC has been presented. The only observable difference between the two routes of drug administration was the apparent accumulation of microdroplets of radiolabelled cannabinoid in both the lungs and spleen after intravenous administration. These sites of very high radiolabel localization were not present in the lungs and spleen of the mice after oral administration of the drug, implying that the accumulation in the two tissues after intravenous injection resulted from the insolubility of the drug, that is, the inability of the drug to remain in solution in the blood. No differences were seen in the overall pattern of $\triangle^9$-THC distribution between the pregnant and non-pregnant animals.

Although the drug acts as a psychotomimetic agent, the brain had about the same low radiolabel concentration as the skeletal muscles, and it appears that the distribution within the brain is very uniform. It is also of interest that although no appreciable concentration of cannabinoid can be detected in the foetus by autoradiography, ethyl acetate extraction of the foetal tissue showed that the drug crossed the placenta and entered the foetal tissue. The chorioallantoic placenta showed only a very slight radiolabel accumulation whereas the yolk sac placenta surrounding the foetuses showed appreciable accumulation of cannabinoid in all of the pregnant mice.

The very high concentration of cannabinoid in the corpora lutea was certainly surprising and warrants further study since the action of this class of compounds on follicle development, ovulation and the secretory phase of the corpus lutea has not been reported in the literature. The high concentration of radiolabel in the mammary glands suggests the possibility of a transfer of $\triangle^9$THC and its metabolites from mother to infant via the mother's milk.

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