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Microsomal conjugation of fatty acids to codeine*

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In previous studies performed in our laboratory (Leighty 1973), long-retained unknown cannabinoic metabolites were detected in the liver, spleen, fat and bone marrow of rats 15 days after a single intravenous or chronic intraperitoneal injection of ^{14}C-Δ^8-tetrahydrocannabinol (^{14}C-Δ^8-THC) or ^{14}C-Δ^9-tetrahydrocannabinol (^{14}C-Δ^9-THC). Δ^8-THC and Δ^9-THC are psychoactive components in marihuana. These unknown metabolites comprised at least 80 percent of the cannabinoids detected in these tissues after 15 days. Subsequent studies (Leighty et al 1976) identified these long-retained cannabinoic metabolites as conjugates of palmitic, stearic, oleic, and linoleic acids.

An in-vitro rat liver coenzyme A fortified microsomal system was later developed in our laboratory that could produce the above fatty acid conjugated cannabinoids from the primary hydroxylated Δ^9-THC metabolite 11-OH-Δ^9-THC (Leighty 1979a) and other cannabinoids (Leighty 1980a).

The present studies were undertaken to determine if codeine, which has a secondary hydroxyl group in its structure, could also be conjugated to fatty acids in our in-vitro rat liver coenzyme A fortified microsomal system.

Materials and methods

Codeine (Mallinckrodt) and [1(m)-^{3}H]codeine (Amersham Searle) were obtained commercially. Palmitoylcodeine, to be used as a reference standard, was synthesized in our laboratory from codeine using palmitoylchloride in pyridine and standard synthesis procedures.

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For in-vitro conjugation to fatty acids 1 mmole of codeine or 100 μmoles of [^{3}H]codeine were incubated in a 37 °C metabolic shaker for 2 h in 25 ml of the rat liver coenzyme A fortified microsomal incubation mixture previously described (Leighty 1979a). The mixture was lyophilized and then extracted three times with 50 ml of chloroform. The solvent was removed on a flash evaporator and the residue resuspended in a small amount of chloroform. The chloroform suspension was then partially purified using thin-layer chromatography (t.l.c.) and high-pressure liquid chromatography (h.p.l.c.).

For t.l.c. aliquots of the chloroform suspension were spotted on silica gel plates (K1, Whatman) and developed in methanol–ammonia (99·5:0·5). Palmitoylcodeine and codeine were spotted on the same plate as reference standards and detected by spraying with acidified iodoplatinate. In this t.l.c. system, codeine has an R_F of 0·54 and palmitoylcodeine an R_F of 0·64. When the initial parent compound was [^{3}H]codeine, a small

Table 1. Diagnostic ions produced by CI and/or EI mass spectrometric analyses of palmitoylcodeine standard, codeine and a h.p.l.c. fraction of extract of microsomal system containing codeine.

	CI		EI	
Compound	(MH+)	Prominent fragment ions	(MH+)	Prominent fragment ions
Palmitoylcodeine standard	538	282	537	282, 229, 162, 124
Codeine			299	229, 162, 124
H.p.l.c. fraction of microsomal extract	538	282	537	282, 229, 162, 124

section of the plate at R_F 0·64 was counted for radioactivity, and the remainder of the t.l.c. R_F 0·64 section scraped, eluted with methanol, dried on a flash evaporator, and resuspended in chloroform. When the parent compound was not radioactive, the same procedure was followed except that the entire t.l.c. R_F 0·64 section was scraped and eluted with methanol.

The t.l.c. eluates were further purified by h.p.l.c. (DuPont 830 Liquid Chromatograph) using the following conditions: Whatman Partisil 10 standard PXS 10/50 column (50 cm × 4·6 mm × 6·4); 50 °C; 1·0 ml min^{-1} flow; chloroform–heptane (80:20) isocratic solvent; 254 UV detector. In this h.p.l.c. system, palmitoylcodeine has a Rt of 11·0 min. The peak areas corresponding to palmitoylcodeine were collected, dried under N_2 and then analysed by mass spectrometry for structural identification.

All mass spectra were collected on a Finnigan 4000 mass spectrometer interfaced to an Incos 2300 series data system. The gas chromatography (g.c.) column used for both chemical ionization (CI-m.s.) and electron impact mass spectrometry (EI-m.s.) consisted of a 0·6 m × 2 mm glass column packed with 1% OV-17. The column temperature was programmed from 180 °C at 6 °C min^{-1}.

The operating parameters for CI-m.s. were: ionizing potential 150 eV; ion source temperature 230 °C, and g.c. carrier gas (CH_4) flow of 17 ml min^{-1}. The reagent gas consisted of a mixture of methane and ammonia (ca 8:1) at a total ion-source pressure of approximately 5×10^{-5} torr.

The operating parameters for EI-m.s. were: ionizing potential 70 eV; ion source temperature 230 °C; and carrier gas (He) flow of 30 ml min^{-1}.

Results and discussion

T.l.c. analyses of the extracts of the microsomal mixtures showed spots at the R_F (0·64) of the palmitoylcodeine standard. Subsequent h.p.l.c. analyses of eluates of the t.l.c. spots showed a peak or peaks at or close to the Rt (11·0 min) of palmitoylcodeine. When [^{3}H]-codeine was the microsomal substrate radioactivity was also detected at the t.l.c. spot and h.p.l.c. retention time of palmitoylcodeine.

CI and/or EI mass spectrometric analyses of the h.p.l.c. peaks, the palmitoylcodeine standard, and codeine are shown in Table 1. The ion at *m/z* 538 in the CI mass spectrum of the palmitoylcodeine standard corresponds to the molecular ion (MH^+) which is consistent with esterification of codeine with palmitic acid. The ion at *m/z* 282 is in agreement with the loss of the fatty acid moiety. The EI mass spectrum of the palmitoylcodeine standard showed high intensity ions at *m/z* 537 and 282 indicative of the molecular ion (M^+) and (M^+-fatty acid), respectively. The fragment ions at *m/z* 229, 162, and 124 are characteristic as shown of the EI fragmentation of codeine. The diagnostic ions in the CI and EI mass spectra of an h.p.l.c. fraction of the microsomal extract are consistent with those shown for the standard indicating that palmitoylcodeine was produced in the microsomal system from codeine. Other CI spectra of the h.p.l.c. peaks showed molecular ions (MH^+) at *m/z* 566, 564, and 562 consistent with conjugation of codeine with stearic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) fatty acids.

Ions were also observed at *m/z* 555 and 583 corresponding to the molecular ions $M(NH_4)^+$ of codeine esterified palmitate and stearate, respectively.

These studies thus show that fatty acids can be conjugated in-vitro to codeine, a compound with a secondary hydroxyl group, as well as to a compound such as 11-OH-Δ^9-THC (Leighty 1979a) which has a primary hydroxyl group in its structure. Additional studies are needed to determine if these codeine fatty acid conjugates are also produced and retained in-vivo in animals.

It has not as yet been determined if fatty acid conjugates of Δ^9-THC are themselves psychoactive. Our laboratory, however, has shown that the fatty acid Δ^9-THC conjugate 11-palmitoyloxy-Δ^9-THC can be hydrolysed with cholesterol esterase and a lipase to psychoactive 11-OH-Δ^9-THC (Leighty 1979b). We have also shown that 11-palmitoyloxy-Δ^9-THC affects the metabolism of a substrate requiring cytochrome P-448 (Leighty 1979c). Recent studies in our laboratory have shown that the drug phencylidine (Leighty & Fentiman 1980b) and the pesticide DDT (Leighty et al 1980c) are also conjugated to fatty acids in our in-vitro system. DDT was also shown to be conjugated to fatty acids in-vivo in male and female rats and retained in their livers and spleens. Other tissues of the rats were not analysed.

Thus, the phenomenon of fatty acid conjugation to certain drugs, pesticides, or other foreign compounds may be a mechanism by which these compounds are retained in the body and exert certain of their beneficial or harmful effects.

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