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β-Diethylaminoethyldiphenylpropylacetate (SKF 525-A) and 2,4-dichloro-6-phenylphenoxyethylamine·HBr (DPEA) inhibition of fatty acid conjugation to 11-hydroxy-Δ^9-tetrahydrocannabinol by the rat liver microsomal system*

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An *in vitro* rat liver coenzyme A fortified microsomal enzyme system that could conjugate certain long-chain fatty acids to 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC) was developed recently by our laboratory [1]. We [2] had previously identified these fatty acid conjugated cannabinoids from both *in vitro* and *in vivo* studies as primarily palmitic, stearic and lesser amounts of C_{18}-unsaturated fatty acid conjugates of Δ^9-tetrahydrocannabinol (Δ^9-THC) and Δ^8-tetrahydrocannabinol (Δ^8-THC). In earlier *in vivo* studies [3], we also found that these fatty acid conjugated cannabinoic metabolites comprised at least 80 per cent of the radioactive cannabinoids retained in the liver, spleen, fat and bone marrow of rats 15 days after an acute intravenous or chronic intraperitoneal injections of [^{14}C]-Δ^8-tetrahydrocannabinol or [^{14}C]-Δ^9-tetrahydrocannabinol. *In vivo* metabolites of cannabinol have also been identified as fatty acid conjugates [4]. Other studies [5, 6] have shown that cholesterol can be conjugated to fatty acids *in vitro* by a similar coenzyme A microsomal system. Thus, the same metabolic pathway in microsomes may be involved in the esterification of cholesterol and 11-hydroxy-Δ^9-THC.

The present studies evaluate the effects of β-diethylaminoethyldiphenylpropylacetate (SKF 525-A) and 2,4-dichloro-6-phenylphenoxyethylamine·HBr (DPEA) on the *in vitro* microsomal fatty acid conjugating system. These compounds are known inhibitors of the classical hepatic microsomal mixed-function oxidase system which is involved in the metabolism of a variety of drugs. For each evaluation, 1 ml of 0.1 M sodium phosphate buffer (pH 7.0), containing 2 μmoles coenzyme A, 10 μmoles ATP and 10 μmoles $MgCl_2 \cdot 6H_2O$, was added to a 16 × 125 mm glass test tube. To this was added 0.5 ml of twice washed microsomes (4 mg protein/ml), 1 μmole [^{3}H]-11-OH-Δ^9-THC in 20 μl ethanol, and 0.5 ml buffer containing either 2, 4 or 8 μmoles SKF 525-A or DPEA. The tubes were incubated in a 37° metabolic shaker for 1 hr and then lyophilized, extracted and evaluated for [^{3}H]-11-palmitoyloxy-Δ^9-THC ([^{3}H]-11-palm-Δ^9-THC) by thin-layer chromatography (t.l.c.) separation and counting of the radioactive t.l.c. sections as described previously [1]. A buffer control, containing everything except microsomes, was also evaluated at the same time as the test samples.

Table 1 shows that both SKF 525-A and DPEA inhibit, at approximately the same percentage, the production of 11-palm-Δ^9-THC from 11-OH-Δ^9-THC. The 2×10^3 M concentrations of SKF 525-A and DPEA, needed for approximately 50 per cent inhibition of this coenzyme A fortified rat microsomal system, were much higher than that usually needed for 50 per cent inhibition of substrates metabolized *in vitro* in the commonly used NADP fortified rat microsomal system [7]. The concentrations were, however, close to that used in microsomes of rabbits and mice for 50 per cent inhibition of aromatic hydroxylation of aniline [7]. It has been reported [8–10] that many drugs, including SKF 525-A, bind nonspecifically to liver microsomal proteins or phospholipids and that effective concentrations and inhibitory potencies of the drugs depend on experimental conditions. The increased concentrations of SKF 525-A and DPEA needed for 50 per cent inhibition of conjugation of fatty acids to 11-OH-Δ^9-THC in our system may be valid or may be due to differences in volumes, microsomal pro-

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Table 1. Effect of SKF 525-A and DPEA on the microsomal *in vitro* production of 11-palmitoyloxy-Δ^9-THC from 11-hydroxy-Δ^9-THC

[^{3}H]-11-OH-Δ^9-THC Incubation system	Total radioactivity in t.l.c. sections* (R_f 0.17–0.34) (d.p.m.)	Radioactivity in 11-palm-Δ^9-THC t.l.c. section* (R_f 0.34) (d.p.m.)	% of Total radioactivity in 11-palm-Δ^9-THC t.l.c. section	Increase in % of radioactivity in 11-palm-Δ^9-THC section over buffer control	% Inhibition
Buffer control (no microsomes)	2428 ± 43	607 ± 16	25.0		
Microsomes	3108 ± 116	1930 ± 114	62.1	37.1	
Microsomes + inhibitor ($\times 10^{-3}$ M)					
SKF 525-A					
1	3770 ± 242	2109 ± 148	55.9	30.9	16.7
2	3329 ± 170	1414 ± 44	42.5	17.5	52.8
4	4691 ± 49	1794 ± 23	38.2	13.2	64.4
DPEA					
1	5665 ± 156	3220 ± 34	56.8	31.8	14.3
2	4206 ± 87	1877 ± 65	44.6	19.6	47.2
4	3506 ± 38	1286 ± 35	36.7	11.7	68.5

* Average of five evaluations ± S.E.M.

tein, pH, or other experimental conditions. It does appear from our studies, however, that SKF 525-A and DPEA not only inhibit enzymes of the mixed function oxidase system involving cytochrome P-450 but also those involved in the conjugation of fatty acids to 11-OH-Δ^9-THC and possibly other hydroxylated compounds, such as cholesterol, which require coenzyme A and ATP in the system. Since little is known about this liver microsomal esterase system, the knowledge that it can be inhibited by SKF 525-A and DPEA may be useful in future studies on the metabolism of certain drugs and other hydroxylated compounds.

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Effects of compound 48/80 on dextran-induced paw edema and histamine content of inflammatory exudate

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It is well known that a local injection of dextran into the hind paw of the rat produces local edema. Pharmacological analysis using antagonists or depletors of histamine or of 5-hydroxytryptamine (5-HT) suggests that histamine and 5-HT are involved in the edema formation [1–3]. We reported recently that the highest concentration of histamine in the exudate collected from the swollen rat paw after injection of dextran occurred prior to the peak of edema, and that the concentration of the amine correlated with the severity of edema [4].

As to the fate of histamine released into the inflammatory site, the histamine is thought to disappear by diffusion into the circulation, as well as by enzymatic inactivation. Several studies suggest that putative mediators of inflammation diffuse into blood [5] or lymph [6–9] from the inflammatory site. Horakova and Beaven [5] have demonstrated clearly that appreciable amounts of released histamine diffuse into the circulation after thermal injury of the rat paw. Such a diffusion of released histamine may also occur during dextran-induced paw edema.

This short communication describes two subjects: (a) the effects of pretreatment of rats with the histamine liberator, compound 48/80, on edema formation and on the histamine content of the exudate and of paw cutaneous tissue after local injection of dextran; and (b) the determination of histamine content in blood plasma from the abdominal aorta and the saphenous vein after local injection of dextran.

Male Sprague–Dawley rats (Charles River Japan Inc., Atsugi, Japan), weighing 140–160 g, were used. Dextran was supplied by the Meito Sangyo Co., Nagoya, Japan. Histamine dihydrochloride was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Compound 48/80 was a product of the Sigma Chemical Co., St. Louis, MO.

Rats were injected intraperitoneally with compound 48/80, 1 mg/kg, four times during 24 hr; control animals received saline instead of compound 48/80. Dextran (average molecular weight 65,000) was dissolved in saline in a concentration of 4.0% (w/v), and 0.05 ml of this solution was injected into the subplantar region of one hind paw 2 hr after the last dose of compound 48/80. The paw volume was measured by a volume differential method before any injection and at various times after injection of dextran. Immediately after the animals were decapitated, incisions about 1 cm long were made on the dorsal and ventral skin of the paw. Without any squeezing, the exudate was then collected with capillary tubes (Propper Manufacturing Co., Inc., Long Island, NY). Pieces of cutaneous tissues, about 8 mm diameter, were obtained from the dorsal (two samples) and ventral (one sample) surfaces of the paw [5]. The tissue samples consisted of skin and the underlying soft tissue.

In another experiment, the concentration of histamine in plasma was determined after local injection of dextran or saline. Under ether anesthesia, blood was taken from the abdominal aorta and the saphenous vein using a needle and a plastic syringe containing 1/10 volume of 1.5% EDTA in saline. Plasma was obtained by centrifuging the blood at 4° at 2200 *g* for 20 min.

Histamine in exudate, paw tissue and plasma was determined by the extraction and fluorometric procedure of Shore *et al.* [10]. The concentrations of histamine in the exudate and plasma are expressed as μg/ml and ng/ml, respectively. The total amount of histamine in the exudate (μg) was calculated by multiplying the concentration of histamine in the exudate (μg/ml) by the increase in paw volume (ml). The histamine content of the paw cutaneous tissue is expressed as μg/g of wet tissue. All values of the amine in this paper are expressed in terms of the free base. The degree of paw edema is expressed as a percentage increase in paw volume relative to the initial volume.

The time course of paw edema formation and the his-