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INFLUENCE OF CHRONIC ORAL INTAKE OF CANNABIS EXTRACT ON OXIDATIVE AND HYDROLYTIC METABOLISM OF XENOBIOTICS IN RAT

PEEYUSH KHANNA, M. B. GUPTA,† G. P. GUPTA,† G. G. SANWAL‡ and BASHEER ALI§

Departments of Biochemistry and †Pharmacology & Therapeutics, King George's Medical College, Lucknow-226003; and ‡Department of Biochemistry, University of Lucknow, Lucknow-226001 (U.P.), India

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Abstract—Dietary intake of petroleum ether extract of cannabis leaves by rats in doses of 158, 250 and 500 mg/kg in the first, second and third week, respectively, caused selective induction of hepatic microsomal carboxylesterases/amidases without affecting the renal hydrolytic activity. Acetanilide *N*-deacetylase, *p*-nitrophenylacetate (NPA) esterase and acetylsalicylic acid (ASA) esterase I and II (active at pH 5.5 and 7.4) were stimulated 125, 64, 82 and 60%, respectively, whereas the activities of procaine esterase and acetylaminofluorene (AAF) *N*-deacetylase remained unaltered. The hydrolysis of acetylcholine was also unchanged. Upon withdrawal of treatment microsomal hydrolytic activity receded to basal levels within 7 days. Curiously though, the two-fold induction of thiacetazone *N*-deacetylase (118%), a cytosolic hydrolase, remained largely undiminished (62%). An appraisal of the hepatic cytochrome P450 mediated oxidative metabolism revealed approximately three-fold induction of aromatic hydrocarbon hydroxylase (AHH) metabolizing benzo(*a*)pyrene whereas the N-demethylation of aminopyrene was unaffected. These activities were restored to normal when resin administration was discontinued.

In vitro and acute *in vivo* studies with purified cannabinoids namely Δ^1-THC¶, Δ^8-THC, Δ^9-THC, CBD and CBN [1] reveal a substrate specific inhibition of hepatic microsomal mixed function oxidases (MFO) [2–5]. In contrast, enzyme activity is selectively enhanced, often with a concomitant increase in cytochrome P450 levels, when individual cannabinoids are administered in repeated doses to rats [6–8]. This conflicting picture, however, fails to relate with the nature of abuse of cannabis by humans which precludes the possibility of any specific component of the herb independently influencing the metabolism of xenobiotics.

An important alternative to oxidative metabolism is the well characterized hydrolytic pathway for the biotransformation of xenobiotics possessing peptide, ester or amide linkage [9]. With the exception of a few cytosolic enzymes, cellular carboxylesterases/amidases of B-type are predominantly microsomal [10, 11]. There are only sporadic reports dealing with the effect of cannabinoids on hydrolytic metabolism. It has been demonstrated that CBD and Δ^1 and Δ^9 isomers of THC exercise a depressant influence on testicular naphthyl esterase and cholesterol esterase both *in vitro* and *in vivo* [12–14].

These observations prompted us to examine the alterations in B-type hydrolytic metabolism upon oral consumption of crude petroleum ether extract of cannabis leaves (given in diet) for 3 consecutive weeks. ASA (Aspirin, an analgesic), procaine (a local anaesthetic), thiacetazone (an antitubercular drug), acetanilide (a model drug), NPA (a nonspecific substrate) and AAF (a carcinogen) were selected as substrates for hepatic and renal B-esterases in rat [15–17]. In order to discriminate the responses of B-esterases from those of cholinesterases the hydrolysis of acetylcholine was assayed under similar experimental conditions. Parallel studies were also undertaken to evaluate the status of MFO mediated metabolism of benzo(*a*)pyrene and aminopyrene in liver.

MATERIALS AND METHODS

Bovine serum albumin, procaine, acetanilide, acetylaminofluorene, acetylcholine chloride, aminopyrene, benzo(*a*)pyrene, glucose-6-PO_4 and NADP were supplied by the Sigma Chemical Co. (St Louis, MO, U.S.A.). Thiacetazone was a gift from Dey's Medical Stores (Manufacturing) Ltd, Calcutta. Aspirin in pure form and all other chemicals used were of analytical grade. All reagents were prepared in deionized water.

A crude extract of the dried leaves of cannabis [18] was prepared by percolating 50 g powdered leaves in approximately 500 mL petroleum ether solvent for 24 hr. The petroleum extract was evaporated to obtain a thick viscous residue. Male albino rats of 90–100 g were acclimatized to a special glucose–gram flour, semi-solid diet for 1 week prior to the commencement of treatment. The diet contained 9 g gram flour, 900 mg dehydrated milk

§ Address all correspondence to: Dr Basheer Ali, Department of Biochemistry, King George's Medical College, Lucknow-226003 (U.P.), India.

¶ Abbreviations: THC, tetrahydrocannabinol; CBD, cannabidiol; CBN, cannabinol; MFO, mixed function oxidases; ASA, acetylsalicylic acid; AAF, acetylaminofluorene; AHH, aromatic hydrocarbon hydroxylase; NPA, p-nitrophenylacetate.

and 100 mg glucose suspended in 20 mL water. Weighed amounts of the cannabis resin mixed in the diet were made available to rats *ad lib* in doses of 200, 400 and 800 mg/kg for the first, second and third week, respectively. The quantity of residue consumed per animal was calculated from the food left uneaten at the end of 24 hr. The average daily intake of the resin was found to be 158, 250 and 580 mg/kg in the first, second and third week, respectively. One batch of treated animals was withdrawn from the cannabis mixed diet 17–18 hr before being killed. Another batch of rats maintained on the same dosage was allowed to live for 7 additional days subsequent to the withdrawal of treatment. The dosage schedule was designed such that all animals were killed by decapitation on the same day facilitating uniformity in experimental conditions. Controls were maintained on the glucose–gram flour diet throughout the study.

Livers and kidneys were removed immediately, chopped, washed thoroughly with ice-cold 1.15% buffered KCl to remove blood, blotted dry and weighed. Twenty per cent homogenates of liver and kidney were prepared in chilled 1.15% KCl buffered with 0.01 M Tris–HCl, pH 7.4 in a Potter-Elvehjem Teflon–glass homogenizer. The liver homogenate was centrifuged at 9000 *g* for 20 min to remove cellular debris, nuclei and mitochondria and subsequently at 101,000 *g* for 60 min to sediment the microsomal pellet which was suspended in an appropriate volume of KCl [15]. The 9000 *g* supernatant, microsomal suspension and cytosolic fraction served as the hepatic enzyme source while the crude kidney homogenate was used as such.

Body weights of animals were recorded just before the commencement of treatment and prior to being killed. Livers were also weighed.

Enzyme assay. Evaluation of hydrolytic metabolism involved the spectrophotometric quantitation of the product formed or substrate disappeared in a given enzyme reaction. ASA esterase I and II active at pH 5.5 and 7.4, respectively, acetanilide *N*-deacetylase, NPA esterase, and AAF *N*-deacetylase were assayed by estimating salicylic acid, aniline, *p*-nitrophenol, and aminofluorene, respectively, produced during the reaction [15–17]. The quantity of substrate that disappeared in a given time was a measure of cholinesterase and procaine esterase activity [17].

Thiacetazone N-*deacetylase.* The reaction mixture in a final volume of 1 mL consisted of 0.1 M Tris–HCl buffer pH 7.0, 0.002 M thiacetazone (in 0.02 mL DMSO) and a suitable aliquot of the cytosolic fraction. The reaction was run for 30 min at 37° and terminated by the addition of DMAB reagent (equal volumes of 1% dimethylamino-benzaldehyde in ethanol and 1 M sodium acetate–HCl buffer, pH 1.4). The resulting yellow color read at 415 nm served as a measure of the rate of formation of *p*-aminobenzaldehyde thiosemicarbazone under the experimental conditions.

Oxidative metabolism was assessed using benzo(*a*)pyrene and aminopyrene as substrates. AHH assay required the fluorometric estimation of the hydroxy metabolites of benzo(*a*)pyrene [19] whereas the rate of N-demethylation of aminopyrene was measured by estimating the formaldehyde generated [20]. In assaying the activity of G-6-PO_4 dehydrogenase, the rate of formation of NADPH per unit time was monitored [21].

The activity of enzymes investigated was linear with respect to time and protein.

Protein was determined by the method of Lowry *et al.* [22] using bovine serum albumin as reference standard. The results were analysed statistically by the Student's *t*-test.

RESULTS

Ingestion of cannabis leaf extract by rat in increasing dosage for 3 consecutive weeks elicited a substrate-specific induction of carboxylesterases/amidases in liver. Under the experimental conditions, hepatic isozymes I and II of ASA esterase were induced 82 and 60%, respectively. Acetanilide *N*-deacetylase and NPA esterase registered a 125 and 64% increase in their activity, respectively. Procaine esterase showed a 20%, albeit statistically insignificant increase while AAF *N*-deacetylase remained unchanged.

Interestingly, cholinesterase also remained unaffected by the treatment. The activity of cytosolic thiacetazone esterase was induced 118%.

Seven days after withdrawal of treatment the activity of microsomal carboxylesterases/amidases had declined to a level comparable to the control. However, the cytosolic esterase metabolizing thiacetazone remained induced by 62% (Table 1).

Unlike liver enzymes the renal hydrolases involved in the biotransformation of ASA, acetanilide, NPA, AAF, thiacetazone and acetylcholine exhibited no change (Table 2).

Prolonged exposure to cannabis effected heterogeneous changes in cytochrome P450 mediated oxidative metabolism of liver. There was an approximately three-fold increase in the rate of hydroxylation of benzo(*a*)pyrene but N-demethylation of aminopyrene remained unmodified (Table 3). Cytosolic G-6-PO_4 dehydrogenase, a NADPH generating enzyme used for MFO assay, registered no effect by this treatment.

As evident from Table 4, the body weights of animals after 21 days treatment with cannabis did not differ significantly when compared to controls. However, a 13.7% increase in the liver–body weight ratio was in consonance with a 35.1% elevation in the microsomal protein content, indicating increased protein synthesis abetting liver enlargement [23].

DISCUSSION

Our studies clearly reveal a selective potentiating effect of cannabis on ASA esterase, acetanilide *N*-deacetylase and NPA esterase in microsomes, and thiacetazone *N*-deacetylase in cytoplasm of rat liver. Different degrees of induction in the hydrolysis of various substrates reaffirm the possible existence of heterogenous multiple forms of B-esterases in mammalian liver [24]. Procaine esterase exhibited only a modest increase while AAF *N*-deacetylase, none at all. It is interesting to note that such treatment failed to disturb hepatic cholinesterase.

Table 1. Effect of cannabis intake on hepatic carboxylesterases/amidases in rat

	Enzyme activity (nanomole product formed or substrate disappeared/min/mg protein)		
		Treated for 21 days	
Substrate*	Control	Killed 17–18 hr after the discontinuation of the resin-mixed diet	Killed 7 days after the discontinuation of the resin-mixed diet
ASA (pH 5.5)	84 ± 5.0	153 ± 13.2†	109 ± 8.8†
(pH 7.4)	66.6 ± 4.5	105 ± 9.2†	73 ± 4.7
AAF	0.78 ± 0.07	0.75 ± 0.06	0.79 ± 0.06
Acetanilide	16.2 ± 1.16	36.45 ± 2.5†	15.9 ± 1.1
NPA	2158 ± 180	3540 ± 242†	2331 ± 167
Procaine	1.12 ± 0.08	1.33 ± 0.10	1.10 ± 0.09
Acetylcholine	3.63 ± 0.27	4.04 ± 0.19	3.78 ± 0.19
Thiacetazone*	0.112 ± 0.007	0.24 ± 0.02†	0.18 ± 0.01†

All values represent means ± SE obtained from five to six animals.
* Hydrolysis of thiacetazone was performed in the cytoplasm whereas that of the remaining substrates in microsomes.
† $P < 0.01$.

Table 2. Effect of cannabis intake on renal carboxylesterases/amidases in rat

	Enzyme activity (nanomole product formed or substrate disappeared/min/mg protein)		
		Treated for 21 days	
Substrate*	Control	Killed 17–18 hr after the discontinuation of the resin-mixed diet	Killed 7 days after the discontinuation of the resin-mixed diet
ASA (pH 5.5)	35.5 ± 1.35	42.0 ± 2.17	35.0 ± 1.33
(pH 7.4)	27.3 ± 1.47	30.2 ± 1.34	28.0 ± 0.89
AAF	0.155 ± 0.006	0.190 ± 0.010	0.155 ± 0.012
Acetanilide	3.26 ± 0.22	3.51 ± 0.30	2.61 ± 0.25
NPA	1130 ± 92	1208 ± 84	933 ± 51
Acetylcholine	1.20 ± 0.06	1.41 ± 0.07	1.18 ± 0.05
Thiacetazone*	0.04 ± 0.002	0.038 ± 0.005	0.037 ± 0.003

All values represent means ± SE obtained from five to six animals.
* Hydrolysis of all substrates including thiacetazone was performed in crude homogenate of kidney.

Table 3. Effect of cannabis intake on hepatic microsomal monooxygenases

		Treated for 21 days	
Enzyme	Control	Killed 17–18 hr after the discontinuation of the resin-mixed diet	Killed 7 days after the discontinuation of the resin-mixed diet
Benzo(*a*)pyrene hydroxylase (pmol hydroxy benzo(*a*)pyrene formed/min/mg protein)	3.02 ± 0.2	9.2 ± 0.67†	2.3 ± 0.16
Aminopyrene *N*-demethylase (pmol HCHO formed/min/mg protein)	370 ± 28	442 ± 25	441 ± 30

Values represent means ± SE obtained from five to six animals.
* $P < 0.001$.

Table 4. Effect of cannabis treatment on microsomal protein, body weight and liver weight of rat

	Control	Treated for 21 days: Killed 17–18 hr after the discontinuation of resin-mixed diet	Treated for 21 days: Killed 7 days after the discontinuation of resin-mixed diet
Microsomal protein (mg/g liver)	29.9 ± 1.06	40.5 ± 1.95*	26.6 ± 1.0
% of initial body weight	115 ± 7.3	118 ± 6.7	117 ± 9.0
% of liver–body weight ratio	3.58 ± 0.18	4.07 ± 0.008†	4.30 ± 0.22†

Values represent means ± SE obtained from five to six animals.
* $P < 0.01$, † $P < 0.05$.

Upon withdrawal of treatment, the inductive effect on microsomal hydrolases gradually ebbs away till it becomes undetectable 7 days later. However, a marked stimulation of the cytosolic thiacetazone esterase persists longer than its microsomal counterparts. These observations lend credence to the view that cytosolic hydrolases are controlled by factor(s) distinct from those regulating the activity of microsomal hydrolases.

Curiously, resin consumption by rat did not evoke any alteration in the capacity of kidney to metabolize esters and amides. Our results indicating non-inducible forms of renal esterases/amidases are in good agreement with those reported earlier where phenobarbital [16], DDT [25] and tobacco [17] administration to rat induced hepatic esterases/amidases but did not affect the enzymes of extrahepatic tissues. These findings suggest that extra-hepatic B-esterases are probably non-inducible in nature.

Prolonged exposure to the crude cannabis extract causes approximately three-fold induction of hepatic AHH without modifying the activity of aminopyrene *N*-demethylase. However, with a decline in the availability of the drug once the treatment is halted, activity of AHH returns to its former level within 7 days. These observations appear in consonance with the reports of a significant induction of hepatic as well as pulmonary benzo(*a*)pyrene hydroxylase when a single dose of Δ^9-THC is administered to rats [26]. Another study has shown that treatment of rats with Δ^9-THC and its metabolites for three successive days causes activation of the rate of biotransformation of benzo(*a*)pyrene while depressing the activity of aminopyrene *N*-demethylase [7]. It seems likely that cannabinoids which have greater access to the microsomal enzyme system owing to their high lipid solubility [27], selectively modify the *de novo* synthesis of microsomal MFO. A recent study has even reported the purification and characterization of a CBD-inducible cytochrome P450 isozyme from mouse hepatic microsomes [28].

The current study demonstrates that cannabis is a selective inducer of B-esterases as well as cytochrome P450 dependent oxidative enzymes. Its interference with the metabolism might influence the pharmacological and toxicological manifestations of drugs and other xenobiotics in individuals habituated to the use of cannabis.

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