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METABOLISM OF HARMALINE IN RATS*

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Abstract—The distribution and metabolic fate of [^{3}H] harmaline-HCl were studied in rats. Thirty min after subcutaneous injection, high radioactivity was found in the small intestine, liver, adrenals, kidneys and lungs. A rapid turnover and elimination was evident after the first hour, as most of the tissues, except the liver, kidneys and intestines, had decreased nearly 50 per cent in levels of radioactivity. About 40 per cent of the harmaline was bound to human serum or rat serum proteins *in vitro*. The blood levels, however, were low at all times *in vivo*. The peak concentration in the brain occurred at 1 hr postinjection. The major route of excretion of harmaline and its metabolites was through the kidneys; a total of 62 per cent of the injected dose was excreted in the urine during 96 hr as compared to only 11·5 per cent in the feces over the same period. The major fate of harmaline in rats was demethylation to form harmalol, which was predominantly excreted as the glucuronide conjugate. Six to 10 per cent of the radioactivity was identified as the sulfate conjugate of harmol, which was formed by the dehydrogenation of harmalol. During the first 8 hr, unchanged harmaline in the urine amounted to about 25 per cent; however, this decreased to only 7 per cent during the 8–24 hr period.

AS EARLY as 1841, Goebel[1] reported the presence of alkaloid harmaline, 7-methoxy-3,4-dihydro-β-carboline, in the seed and root of *Penganum harmala*. This plant is native to the South Russian steppes, Syria and India. Harmaline is also present in several different species of *Banisteria caapi* which are indigenous to South America. It is an inhibitor of the enzyme, monoamine oxidase, a potent serotonin antagonist and a known hallucinogen.[2] Gunn,[3] in 1935, observed the production of tremors with clonic convulsions in mammals by harmaline followed by a depressant state of central origin. Harmalol, the demethylated analog of harmaline, on the other hand, causes progressive paralysis of the CNS without producing convulsions.

In view of the different pharmacological actions the two compounds possess and the reported formation of harmalol from harmaline *in vivo*,[4] we have undertaken the detailed study of the metabolic fate and an autoradiographic study of the distribution of harmaline in rats using a tritium-labeled compound.

MATERIALS AND METHODS

Compound

Harmaline hydrochloride dihydrate (Regis Chemical Company) was labeled with tritium by a tritium–hydrogen exchange reaction.[5] Labile tritium atoms were removed until a constant specific activity of 190 μc/mg was achieved. The purity of the radioactive compound was verified by its infrared spectrum and by thin-layer

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chromatography (TLC; Silica gel G). The chromatograms were developed in the systems of *n*-BuOH–HOAc–H_2O (4:1:1) and in *n*-C_3H_7OH–NH_4OH–H_2O (4:1:1). Harmaline-^3H was detected on the plates by autoradiography and ultraviolet fluorescence. Harmalol hydrochloride and harmol hydrochloride were purchased from the Regis Chemical Company.

Tissue distribution

Female Sprague–Dawley rats weighing 120 g were given harmaline-^3H, 40 mg/kg (7·6 mc/kg), subcutaneously. The tissues of three animals from the specified time interval were dissected, homogenized in 10 parts of 75% aqueous methanol and centrifuged. The supernatant was assayed for tritium by liquid scintillation (liquid scintillation fluor: 4 g PPO,* 50 mg POPOP* and 70 g naphthalene per l. toluene). All values were corrected for 10 per cent efficiency (channel ratio) and recovery. The blood samples were lyzed with water, decolorized with 10% H_2O_2 in methanol and heated in closed counting vials, first at 45° for 15 min and then at 70° for 2 hr with 1 N NaOH. After neutralizing with 2-ethylhexanoic acid and the addition of a 20% solution of Beckman Solubilizer (BBS-3) in liquid fluor, the radioactivity was assayed by liquid scintillation.

Total body autoradiography was done as previously described.[6–8]

Excretion

Urine and feces were collected from two groups of three animals maintained for 7 days in metabolic cages with free access to food and water. The urine samples were mixed with methanol and radioactivity was assayed by liquid scintillation. The fecal samples were treated as described for tissues.

Four male rats (300–350 g) were given urethane (1·2 g/kg) intraperitoneally and the bile ducts were cannulated. After subcutaneous injection of harmaline-^3H (40 mg/kg), bile and urine samples were collected for 48 hr and their radioactivities assayed. Saline was administered subcutaneously to prevent dehydration.

Serum protein binding

Serum protein binding was studied *in vitro*, using rat and human serum and utilizing a modification of a procedure described by Saris.[9] Sephadex G-25 and Nytex nylon screen (105 μ, POW Company) were used.

Chromatography

Hydrolyzed and unhydrolyzed urine samples were applied to Whatman No. 1 paper in solvent system A (Table 2) using descending technique. The radioactive bands, after being detected by strip scanning, were cut off and eluted by methanol. The eluants were rechromatographed in solvent systems B and C, and also on Silica gel G plates in solvents D, E and F (Table 2). The radioactivity of the metabolities was determined. Harmaline and its metabolities were identified by comparison to reference compounds for R_f values, ultraviolet fluorescence, spectrophotofluorometry, and color reaction with diazotized sulfanilic acid and Brentamine Fast Blue.

* PPO, 2,5-diphenyloxazole; POPOP, 1,4-bis-2-(4-methyl 5-phenyloxazolyl)benzene.

For enzymatic hydrolysis of conjugates, urine was incubated at 37° in sodium acetate buffer, pH 5·0, with beef liver Ketodase (β-gluduronidase; 500 units/ml) to hydrolyzed glucuronide conjugates. Urine samples of the same time interval were adjusted to pH 5·5 and incubated at 37° overnight with Glusulase (β-glucuronidase and sulfatase, Endo Laboratories, Inc.; 500 units/ml) in sodium acetate buffer.

RESULTS AND DISCUSSION

Distribution of radioactivity in rat tissues at various time intervals after subcutaneous administration of harmaline is tabulated in Table 1. At 30 min postinjection, high radioactivity was found in the small intestine (with contents), liver, adrenals, kidneys

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN RAT TISSUES AT VARIOUS TIME INTERVALS AFTER SUBCUTANEOUS INJECTION OF [^{3}H] HARMALINE-HCl (40 mg/kg)

Tissues	Radioactivity* (μc/g tissue)					
	30 min	1 hr	2 hr	8 hr	24 hr	48 hr
Adrenals	22·7	16·3	8·39	2·47	0·49	0·49
Brain	10·9	11·9	6·66	1·33	0·59	0·54
Heart	6·80	6·07	3·21	1·04	0·49	0·69
Lungs	17·5	14·2	7·84	1·78	0·39	0·49
Muscle	10·7	2·12	1·28	0·69	0·44	0·35
Ovaries	1·80	1·40	0·80	0·10	0·05	0·09
Spleen	9·32	9·77	5·33	1·04	0·69	0·54
Kidney	20·9	18·4	13·0	3·55	1·87	1·13
Liver	26·6	25·7	20·0	3·80	2·32	1·23
Small intestine	34·5	44·4	60·7	25·0	1·23	0·74
Large intestine	5·03	5·03	5·62	69·6	2·17	0·89
Blood†	3·26	3·16	1·78	0·79	0·35	0·35
Plasma†	2·8	2·8	1·92	1·33	0·74	0·54

* Each value represents the mean of three rats.
† These values are expressed as μc/ml.

and lungs, indicating rapid uptake of the compound by these tissues. However, most of rat tissues, except liver, kidneys and intestines, had a 50 per cent decline in radioactivity levels during the second hour after injection. This would seem to suggest a rapid turnover and elimination of harmaline or its metabolities or of both. After 8 hr, the radioactivity in the blood was concentrated in plasma (Table 1), indicating an affinity of harmaline or the metabolities (or of both) for plasma. The binding of harmaline-HCl after 30 min by human serum and rat serum was studied *in vitro* and found to be 40·6 and 38 per cent respectively.

Harmaline entered the brain within 30 min and reached a peak concentration 1 hr after subcutaneous injection, at which time the brain contained radioactivity equivalent to 1·4 per cent of the injected dose; 75 per cent of this was identified as unchanged harmaline.

The autoradiographic data replicated the data obtained by assay of tissue homogenates. Additional sites of accumulation were detected in salivary glands, thymus, bone marrow and retina (Fig. 1, A,B). The accumulation in the retina is of significance

because of the report of Naranjo[2] that harmaline has a direct effect on the retina; this could be a contributing factor to the hallucinogenic effect of harmaline. An indication of the excretory pathway is seen in these sections. At 30 min, a high concentration of radioactivity was evident in the contents of the upper small intestine and in the intestinal wall (Fig. 1,A). At 2 hr, the activity was high throughout the small intestine, but low in the colon; this was also seen in the 24-hr section (Fig. 1,B,C). The high concentration of radioactivity in the stomach at 2 hr seemed to indicate gastric excretion (Fig.1,B). Radioactivity in the kidney was high at all times. In all three sections, the site of injection was consistently very high in radioactivity, demonstrating that the compound was very poorly absorbed.

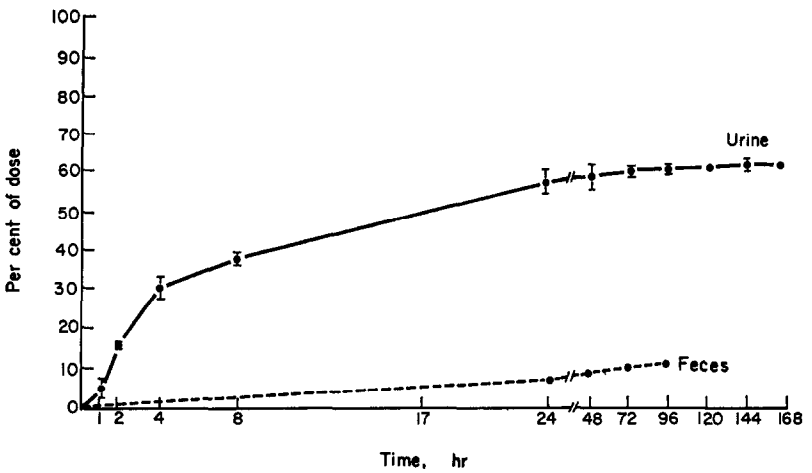

FIG. 2. Excretion of radioactivity in urine and feces after subcutaneous injection of [^{3}H] harmaline-HCl (40 mg/kg). Each point represents the mean of two groups (six rats); the maximum range was 3 per cent.

The rate of excretion of [H^3] harmaline and its metabolities in urine and feces is shown graphically in Fig. 2. A total of 62 per cent of the injected activity was recovered in the urine up to 96 hr as compared to the 11·5 per cent which accumulated in the feces over the same period.

Table 2 shows the characteristics of urinary metabolites of harmaline. When urine samples of several combined intervals, such as 0–2, 2–8, and 8–24 hr were chromatographed on paper, they were separated into six major bands, each of which was rechromatographed on TLC plates in three different solvent systems. Spots 4, 5 and 6 were identified, respectively, as harmalol (7-hydroxy-3, 4-dihydro-β-carboline, the demethylated product), harmaline (unchanged), and harmol (7-hydroxy-β-carboline, resulted from demethylation and dehydrogenation) by chromatographic R_f values, ultraviolet fluorescence, color reaction, and fluorescence spectrum against reference standards (Table 2). Harmalol and harmaline were further characterized by the isotope dilution technique.

Upon hydrolysis of the urine with Ketodase, spot 1 was no longer detectable on the chromatogram, and the increase in the radioactivity of spot 4 (harmalol) indicated that spot 1 was the glucuronide conjugate of harmalol.

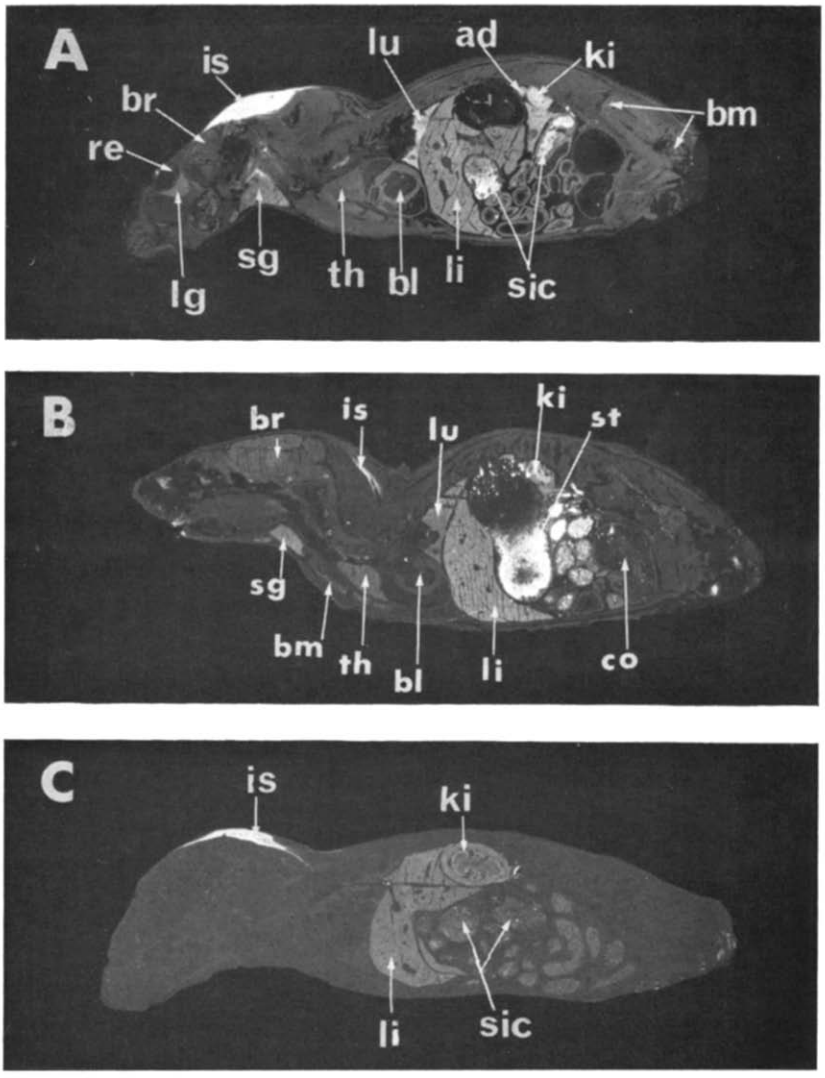

FIG. 1. Distribution of radioactivity (light areas) in rats 30 min (A), 2 hr (B), and 24 hr (C) after injection of [^{3}H] harmaline-HCl subcutaneously. Abbreviations: ad, adrenal; bl, blood in heart; bm, bone marrow; br, brain; co, colon; is, injection site; ki, kidney; lg, lacrimal gland; li, liver; lu, lung; re, retina; sg, salivary gland; sic, small intestinal content; st, stomach; and th, thymus.

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TABLE 2. CHARACTERISTICS OF MAIN URINARY METABOLITIES AFTER SUBCUTANEOUS ADMINISTRATION OF [^{3}H] HARMALINE-HCl (40 mg/kg)

	Metabolite	R_f Solvent system* A	B	C	D	E	F	u.v. Fluorescence†	Colour reaction‡ A	B	Fluorescence spectra§ λa (mμ)	λe (mμ)
1	Glucuronide conjugate	0·28	0·28	0·53	0·00	0·11	0·00	B				
2	Sulfate conjugate	0·47	0·67	0·73	0·11	0·36	0·00	B				
3	Sulfate conjugate	0·52	0·67	0·75	0·26	0·44	0·00	Pu				
4	Harmalol	0·72	0·76	0·79	0·47	0·56	0·00	YB	Red Br	Lt Br	370	480
5	Harmaline	0·80	0·90	0·86	0·69	0·54	0·04	BG	Or Br		380	485
6	Harmol	0·80	0·76	0·86	0·73	0·64	0·24	B-Pu	Red Br	Lt Br	330	430

* Solvent systems: A, *n*-butanol–acetic acid–water (15:3:5); B, *n*-butanol–isopropanol–ammonia–water (3:1:1:1); C, methanol–*n*-butanol–benzene–water (2:1:1:1); D, chloroform–methanol–isopropanol–ammonia (90:10:95:5); E, *n*-butanol–acetic acid–water (4:1:1); F, chloroform–methanol (9:1).

† B, blue; Pu, purple; Y, yellow; G, green.

‡ Color reaction: A, with Brentamine Fast Blue; B, with diazotized sulfanilic acid; Br, brown; Or, orange; Lt, light.

§ Fluorescence spectra were taken in 0·1 N HCl: λa, activation maximum; λe, fluorescence maximum.

Glusulase hydrolysis caused the disappearance of spots 1, 2 and 3. Besides a simultaneous increase in the radioactivity of spot 4, like that which occurred in the Ketodase treatment, a new spot 7, corresponding to the amount of radioactivity in spot 2, appeared with an R_f value of 0·89 in solvent system D (Table 2). Since this sulfate conjugate was present in urine in a very small amount, its characterization was not carried out. Spot 3 was shown to be a sulfate conjugate of spot 6 (harmol) by the shift of radioactivity from spot 3 to spot 6.

The per cent of radioactivity of the urinary metabolities of harmaline at various time intervals is shown in Table 3. The metabolism of harmaline in rats was rapid and extensive. In the first 8 hr, 75 per cent of the compounds present in the urine were the metabolities of harmaline, and the ratio of metabolities to unchanged harmaline increased after 8 hr (Table 3).

TABLE 3. PER CENT OF RADIOACTIVITY OF THE URINARY METABOLITES AT VARIOUS TIME INTERVALS AFTER SUBCUTANEOUS INJECTION OF [H^3] HARMALINE-HCl (40 mg/kg)

Metabolite	Per cent radioactivity*					
	0–2 hr		2–8 hr		8–24 hr	
	Free	Conj.	Free	Conj.	Free	Conj.
Harmaline	26·3	0·00	23·8	0·00	7·34	0·00
Harmalol	0·87	60·5	0·82	68·9	3·07	81·4
Harmol	0·35	9·46	0·22	5·52	0·23	6·11
Unidentified metabolite	trace	1·77	trace	1·16	trace	0·81

* These values were corrected for loss of radioactivity due to tritiated water. Urine samples on chromatographic paper were assayed for tritium after drying.

Figure 3 illustrates the metabolic pathway of harmaline. Instead of the expected hydroxylation of the C-6 position to form 6-hydroxyharmaline,[10] the major metabolic product of harmaline was the *O*-demethylated product, harmalol, which was excreted almost exclusively as the glucuronide conjugate. There is a considerable difference in the metabolic fate of methoxy-substituted β-carbolines and derivatives in rats. Study of the metabolism of 6-methoxytetrahydro-β-carboline showed that equal quantities of the hydroxylated and the demethylated metabolites were excreted in urine.* Furthermore, Kveder and McIsaac[11] reported that 6-hydroxymelatonin was the sole metabolic product of melatonin, and no demethylated product was found. Whether the position of the methoxy group or the basicity of the compound or both, are the parameters governing the fate of these compounds in rats remains to be studied.

The conversion of harmalol to harmol proceeded through dehydrogenation. The possibility that this aromatic β-carboline could be formed *in vitro* during the handling of urine samples was ruled out. The formation of harmol from harmalol was not observed *in vitro* in the enzyme hydrolysis medium; neither could it be produced on Silica plates by silicic acid.

* B. T. HO, D. TAYLOR, K. E. WALKER and W. M. MCISAAC, to be published.

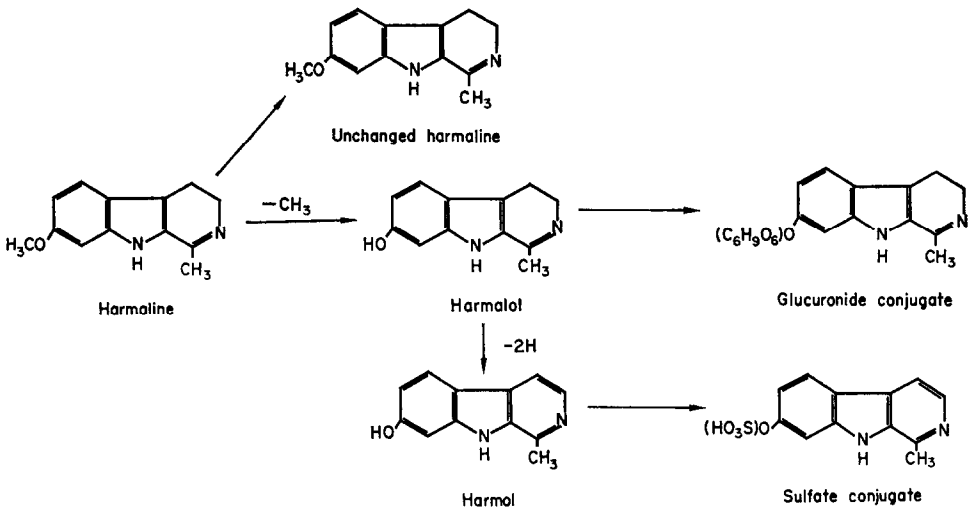

FIG. 3. Metabolic fate of harmaline in rats.

In our studies, neither harmine (7-methoxy-β-carboline) nor harminic acid (6-azaindole-2,3,dicarboxylic acid) was found in the rat urine, as reported earlier.[12] The absence of harmine clearly indicated that harmol could only be derived from harmalol. Traces of harmine were found to be present in urine in our initial studies; however, upon further examination, the presence of harmine was found to be an artifact in the commercial material. After further purification of the tritiated harmaline by sublimation, harmine was no longer detected as a metabolite of harmaline.

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