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Metabolism and Excretion of Normorphine in Dogs

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Abstract □ Normorphine metabolism was studied in dogs given 20 mg of normorphine hydrochloride/kg sc. Free and conjugated normorphine excreted in the urine over 144 hr represented 32 and 32%, respectively, of the administered dose. Eighty percent of the urinary excretion of the drug occurred within 9 hr. One percent of the administered dose was excreted as free normorphine in the feces. The urine was chromatographed on a column. Evaporation of the washing and methanolic effluent yielded a residue, which was purified by crystallization from aqueous methanol. Results of UV and IR studies, elemental analysis, and determination of normorphine and glucuronic acid content established the identity of this metabolite as normorphine 3-glucuronide. Dihydronormorphine and dehydronormorphine were detected with GLC–mass spectrometry as minor metabolites.

Keyphrases □ Normorphine—metabolism and excretion in dogs □ Metabolism—normorphine in dogs □ Excretion—normorphine in dogs □ Narcotic analgesics—normorphine, metabolism and excretion in dogs

A normorphine conjugate was observed in the urine of humans and laboratory animals after administration of normorphine, morphine, and codeine (1–6), but its nature has not been characterized. The excretion of normorphine in the dog, the isolation and characterization of the normorphine conjugate as normorphine 3-glucuronide, and the detection of dihydronormorphine and dehydronormorphine as minor metabolites are discussed in this report.

EXPERIMENTAL

Materials and Methods—Normorphine hydrochloride[1], 20 mg/kg sc, was injected into two female dogs deprived of food for 24 hr and hydrated by intubation with about 500 ml of water. The dogs were housed in individual metabolic cages, and urine and feces were collected for 144 hr.

Normorphine was determined by GLC in the urine and feces, with and without hydrolysis, as described (4). Specimens were hydrolyzed with acid or β-glucuronidase[2] (4). Free and total normorphine were estimated from unhydrolyzed and hydrolyzed specimens, respectively. Conjugated normorphine was calculated as the difference between the total and the free drug.

GLC–Mass Spectrometry—Chemical-ionization mass spectral data were obtained on a gas chromatograph–mass spectrometer[3] as described (7). The mass fragment pattern of obvious GLC peaks was analyzed. In addition, the whole chromatogram was scanned for possible normorphine metabolite ions such as morphine, norcodeine, *N*-hydroxynormorphine, dihydronormorphine, and monohydroxynormorphine.

Isolation of Normorphine Metabolites—An aliquot of the first 24-hr urine was centrifuged and passed through a resin[4] column (2.1 × 40 cm). The column was washed with 150 ml of distilled water and eluted with 300 ml of 2.5% NH_4OH in methanol. The water washing and the methanol eluate were separately evaporated to syrupy residues under reduced pressure in a water bath at 50°. The residues were suspended in 20 ml of 2% NH_4OH and extracted three times with 1,2-dichloroethane containing 30% 2-propanol. The extracts were concentrated for identification of free normorphine metabolites. The aqueous phase was used to isolate conjugated normorphine as described below.

An aliquot of the free base fraction along with authentic normorphine was chromatographed on instant TLC sheets impregnated with silica gel[5], with ethyl acetate–methanol–ammonium hydroxide (17:2:1) as the developing solvent. After a portion of the chromatogram was sprayed with iodoplatinate, three spots appeared with R_f values of 0.6 (pinkish), 0.73, which corresponds to authentic normorphine (bluish purple), and 0.95 (bluish purple). The chromatogram was sectioned horizontally according to the zones 0–75 (R_f 0.3), 76–110 (R_f 0.6), 111–140 (R_f 0.73), and 141–155 (R_f 0.95) mm and eluted with methanol. The eluates were concentrated and analyzed by GLC.

The aqueous phase was evaporated to a syrupy residue. The residue was triturated with methanol to yield methanol-soluble and methanol-insoluble fractions. The methanol-insoluble fraction was recrystallized from aqueous methanol and dried under vacuum, and it yielded 50 mg of snow-white crystalline material. Approximately 60% of the conjugate was isolated from the water washing fractions.

The methanol-soluble fraction was chromatographed on instant TLC sheets impregnated with silica gel[5], with 1-butanol–acetic acid–water (35:3:10) (Solvent A) as the developing solvent. A section of the chromatogram was sprayed with iodoplatinate solution and showed four spots at R_f 0.0, 0.33, 0.6, and 0.9. The chromatogram was sectioned horizontally according to zones 0–50 (R_f 0.33), 51–120 (R_f 0.6), and 121–150 (R_f 0.9) mm and eluted with water. The eluate was concentrated, hydrolyzed with

[1] Merck Sharp & Dohme, Philadelphia, Pa.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Finnigan model 3300.
[4] Amberlite XAD-2 resin, Rohm & Haas Co., Philadelphia, Pa.
[5] Gelman Instrument Co., Ann Arbor, Mich.

Table I—Urinary and Fecal Excretion (Percent of Administered Dose) of Normorphine and Normorphine Conjugate in the Dog

	Urine				Fecal[a]
	Free Normorphine		Conjugated Normorphine		Free Normorphine
Hours	Dog 1[b]	Dog 2	Dog 1	Dog 2	Dog 1
0–3	13.35		4.92		
3–6	4.52		16.29		
6–9	7.65		5.86		
9–12	1.98		0.09		
12–24	3.24		1.73		
0–24	30.74	29.66	24.37	24.95	1.00
24–48	0.56	2.00	5.68	5.19	0.05
48–72	0.23	1.15	1.26	0.95	0.0
72–96	0.03	0.44	0.53	0.24	0.0
96–120	0.04	0.48	0.10	0.36	0.0
120–144	0.0	0.47	0.0	0.32	0.0
Total	31.60	32.33	31.94	32.08	1.05

[a] Conjugated normorphine was not found in the feces. [b] The urinary bladder of Dog 1 was catheterized.

hydrochloric acid or with β-glucuronidase, extracted, and derivatized for analysis by GLC.

RESULTS AND DISCUSSION

Urinary and Fecal Excretion of Normorphine—Free and conjugated normorphine excreted in the urine over 144 hr represented 32 and 32%, respectively, of the administered dose (Table I). Eighty percent of the urinary excretion of free and conjugated drug occurred within 9 hr in one catheterized dog. One percent of the administered drug was excreted in the feces in one dog. The low recovery of normorphine in the feces was probably due to hydrolysis of the normorphine conjugate in the gut and reabsorption[6].

The extent of conjugation of normorphine appears different from that of morphine. More than 50% of excreted normorphine found in the urine of dogs, monkeys, and humans was in the free form (1, 2), whereas only about 10% of excreted morphine found in the urine of the same species was in the free form and 90% was in the conjugated form (4, 8, 11).

Characterization of Normorphine Metabolites—*Normorphine Conjugate*—Acid hydrolysis of the conjugate gave a product identical with normorphine on TLC, GLC, and GLC–mass spectrometry. The conjugate was soluble in acid, slightly soluble in water, and insoluble in methanol and other organic solvents. It was positive with the glucuronide test (12) and negative with the phenolic test (13). It did not melt or decompose below 300°.

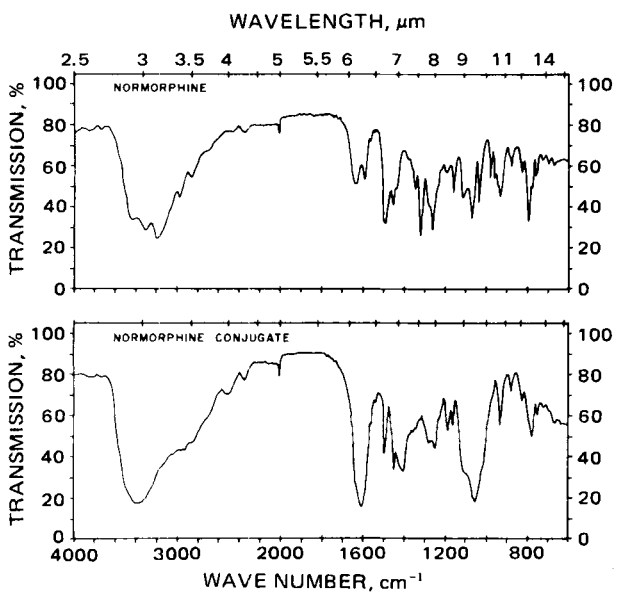

Figure 1—*IR spectrum of authentic normorphine and normorphine 3-glucuronide isolated from dog urine.*

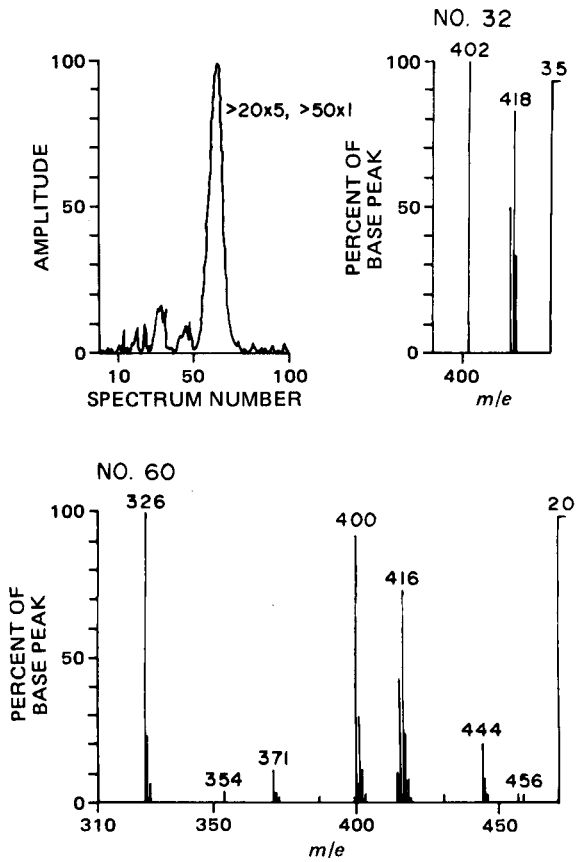

Figure 2—*Integrated total ion current chromatogram of an extract of the hydrolyzed methanol-soluble fraction as the trimethylsilyl derivative. Mass spectrum No. 32 showed characteristics of dihydronormorphine, and No. 60 showed characteristics of normorphine.*

The purified conjugate showed a single spot at R_f 0–0.1 on instant TLC sheets impregnated with silica gel[4] and developed with Solvent A. The R_f value of the conjugate in the methanol eluate was 0.3–0.4; the R_f value appeared to change in the process of purification. The R_f value of the conjugate on paper[7] chromatography, developed with 1-butanol–acetic acid–water (4:1:2), was 0.2.

The IR spectrum of the conjugate as a potassium bromide pellet showed strong absorption bands at 3400, 1610, 1400, and 1070 cm^{-1}, indicating the presence of polyhydroxyl, carbonyl, carboxyl, and ether groups (Fig. 1). The UV spectra of the conjugate in hydrochloric acid medium showed a maximum at 283 nm and a minimum of 256 nm. The $E^{1\%}_{1\,cm}$ was 220. The conjugate in an alkaline medium did not show a bathochromic shift, indicating that the phenolic group was masked. After acid hydrolysis, it did show a bathochromic shift.

Anal.—Calc. for $C_{22}H_{26}NO_9$ (normorphine 3-glucuronide): C, 58.43; H, 5.86; N, 3.13. Found: C, 59.02; H, 5.59; N, 2.79.

The normorphine content of the conjugate was 60.94% as determined by UV and 60.70% by GLC after hydrolysis, extraction, and derivatization. The content of glucuronic acid was 45.5%. The theoretical values for normorphine 3-glucuronide are 60.64% normorphine and 43.39% glucuronic acid.

Detection of Dihydronormorphine—A small peak at *m/e* 418, corresponding to dihydronormorphine as the trimethylsilyl derivative (the amine group was not silylated under the conditions described), was detected in the hydrolyzed eluate of the 0–50-mm zone (R_f 0.3) of the chromatogram of the methanol-soluble portion of the triturated syrupy residue (Fig. 2). Normorphine, like morphine, was expected to be biotransformed to dihydronormorphine since dihydromorphine was found as a minor metabolite of morphine (14).

Detection of Dehydronormorphine—A small peak at *m/e* 558, corre-

[6] Although no data are available on the biliary excretion of normorphine and its conjugate(s), normorphine, like morphine, probably is excreted largely in the bile (8–10).

[7] Waterman No. 1.

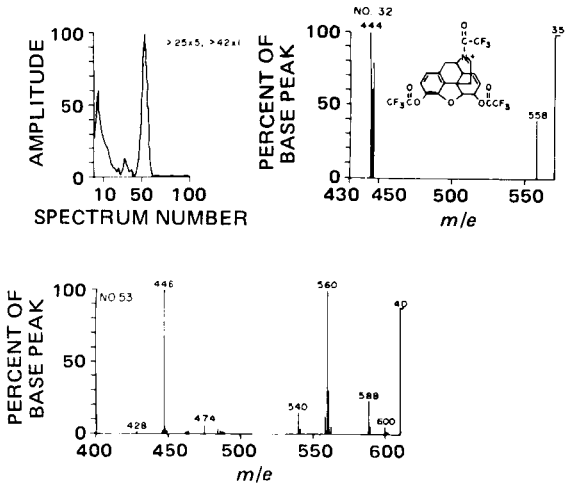

Figure 3—*Integrated total ion current chromatogram of an extract of the free base drug fraction as the trifluoroacetyl derivative. Mass spectrum No. 32 showed characteristics of dehydronormorphine, and No. 53 showed characteristics of normorphine.*

sponding to dehydronormorphine as the trifluoroacetyl derivative, was observed in the eluate of the 140–155-mm zone (R_f 0.9) of the thin-layer chromatogram of the free base drug fraction (Fig. 3). Although there is no direct evidence for the presence of *N*-hydroxynormorphine, dehydronormorphine may have resulted from dehydration of *N*-hydroxynormorphine during purification, derivatization, and GLC analysis. Meperidine *N*-oxide has been shown to undergo dehydration during GLC analysis (15, 16).

Morphine, norcodeine, and codeine were not detected in the free or the conjugated form. This result confirms the reports that the methylation of normorphine does not play a significant role in the metabolism of normorphine (17–20).

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Local Anesthetic Activity and Acute Toxicity of *N*-Substituted 1,2,3,4-Tetrahydro-1- and 2-naphthylamines

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Received August 16, 1976, from the *Department of Pharmacology and Medicinal Chemistry and the Department of Organic Research, Abbott Laboratories, North Chicago, IL 60064.* Accepted for publication September 29, 1977.

Abstract □ Seven *N*-substituted 1,2,3,4-tetrahydro-1- and three 2-naphthylamines were prepared and tested for local anesthetic activity in the rabbit corneal reflex test and the mouse sciatic nerve block test. At 0.1 and 1%, three 1-alkylamino compounds had durations of action comparable to that of tetracaine in the rabbit corneal reflex test and were considerably more potent than lidocaine. The other four 1-alkylamino derivatives were inactive or at best minimally active. The durations of action of 1% concentrations of the three 2-alkylamino compounds were equivalent to that of 1% lidocaine in the corneal reflex test. In the mouse sciatic nerve block test, the three active 1-alkylamino compounds were considerably longer acting than either tetracaine or lidocaine. Three 1-alkylamino and the three 2-alkylamino compounds showed toxicity equal to or greater than lidocaine, while two 1-alkylamino and two 2-alkylamino compounds showed toxicity equal to or greater than tetracaine by the intraperitoneal route in mice. *N*-Heptyl-1,2,3,4-tetrahydro-6-methoxy-1-naphthylamine methanesulfonate was the most promising local anesthetic in these series.

Keyphrases □ 1,2,3,4-Tetrahydro-1- and 2-naphthylamines, *N*-substituted—synthesized, local anesthetic activity and toxicity evaluated □ Local anesthetic activity—various *N*-substituted 1,2,3,4-tetrahydro-1- and 2-naphthylamines evaluated □ Toxicity—various *N*-substituted 1,2,3,4-tetrahydro-1- and 2-naphthylamines evaluated □ Structure-activity relationships—various *N*-substituted 1,2,3,4-tetrahydro-1- and 2-naphthylamines evaluated for local anesthetic activity and toxicity

A series of methoxy-1- and 2-aminoindans and naphthylamines previously was synthesized as potential antiparkinsonian agents (1). When these compounds were tested for dopaminergic activity, some were inhibitors of monoamine oxidase. Subsequently, additional compounds were prepared in which the substituent on the amine was