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COMPARATIVE METABOLISM OF HYDROCODONE IN MAN, RAT, GUINEA PIG, RABBIT, AND DOG

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ABSTRACT

The metabolism of hydrocodone was studied in man, rat, guinea pig, rabbit, and dog. Routes of metabolism included O-demethylation, N-dealkylation, and 6-keto-reduction to the corresponding 6-α- and 6-β-hydroxy metabolites, where each metabolic pathway produces an active metabolite. Mean total recovery of drug and metabolites as percentage of administered dose ranged from a low of 10.6% for the rabbit to a high of 46.8% for the guinea pig; man was intermediate at 25.7%. For man, approximately 70% of the total drug recovered was excreted in the first 24 hr, and the remainder by 72 hr. Considerable species differences were observed in the patterns of metabolism of hydrocodone. Also, stereoselectivity of 6-keto reduction to the β-form was observed for all species in the reduction of hydrocodone and hydromorphone with the exception of the reduction of hydrocodone by man.

Hydrocodone (dihydrocodeinone) (HC)[2] is a semisynthetic congener of codeine and is approximately equipotent to morphine in producing opiate effects (1, 2). Although HC has an addiction liability similar to that of morphine it has been widely used as an antitussive for many years, apparently without serious abuse.

The metabolism of HC was of interest because of the similarity of molecular structure to other narcotic agonists and antagonists for which stereoselective reductions of the C_6-keto group have been reported, such as naltrexone (3), naloxone (4, 5), and hydromorphone (6). The only report in regard to the metabolism of HC describes determination by electron-capture GC of the parent drug in the serum of man and dog as the pentafluorophenylhydrazone derivative (7). An additional peak corresponding in retention time to that of norhydrocodone was observed for dog and to a lesser extent for man. The lack of information concerning the metabolism of HC prompted a study of its biotransformation in man, rat, dog, guinea pig, and rabbit.

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[2]Abbreviations used are: HC, hydrocodone; HM, hydromorphone; 6αHC, 6-α-hydrocodol; 6βHC, 6-β-hydrocodol; 6αHM, 6-α-hydromorphol; 6βHM, 6-β-hydromorphol; GC, gas chromatography; GC/MS, gas chromatography/mass spectrometry; CI, chemical ionization.

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Materials and Methods

Chemicals. All solvents were of reagent grade and were used without further purification. HC and HM were obtained from Knoll Pharmaceutical Co. (Orange, N.J.) and Wm. S. Merrell Co. (Cincinnati, Ohio). 6αHM, 6βHM, and 6βHC were obtained from the Drug Addiction Laboratory, University of Virginia. 6αHC and NC were generous gifts from Dr. Everette May, formerly of the National Institutes of Health, and Dr. J. W. Barnhart, Dow Chemical Co. (Midland, Mich.). All compounds were analyzed by GC/MS for structural verification.

Animals and Subjects. The animals used in this study consisted of one male and one female dog (mongrel beagle, approximately 3 years old), male rats (albino Wistar, 6–9 months), male rabbits (albino New Zealand, 10–12 months) and male guinea pigs (albino Hartley, 6–9 months). The six humans in this study were healthy, adult male federal prisoner volunteers from whom informed consent had been obtained under N.I.H. guidelines. All subjects were drug-free at the time of the study. The age (years) and weight (kg) of each subject were as follows: subject 1, 38, 86.2; subject 2, 33, 118.8; subject 3, 34, 77.1; subject 4, 41, 74.8; subject 5, 31, 74.8; subject 6, 29, 74.8.

Instrumentation. GC analyses were performed in a Varian model 2700 instrument equipped with flame-ionization detectors. The carrier gas used was nitrogen

at a flow rate of 30 ml/min. The twin glass columns (1.8 m × 2 mm I.D.) were packed with 3% OV-17 on 100/120-mesh Gas-Chrom Q. The detector, injector, and column temperatures were maintained isothermally at 275°, 275°, and 250°C, respectively.

GC/MS with CI was used for the detection and measurement of HC, HM, and their respective 6-hydroxy metabolites in the acid-hydrolyzed urine of two human subjects. Analyses were performed by single-ion recording of the pseudomolecular ion produced by methane CI. Cyclazocine was used as an internal standard. A detailed description of the analytical procedure has been reported (8).

Sample Collection and Extraction. The procedures used for urine collection and extraction were the same as those described for the metabolism of HM (6) with the exception that *n*-butyl chloride was used as the solvent for extraction in the GC assay of HC, 6αHC, 6βHC, and NC. Urine samples from each species, with the exception of man, were pooled before analyses for drug and metabolite content. Individual human samples were collected and analyzed. The experiments are summarized in table 1. A therapeutic dose in man and a subtoxic dose in animals were used, which provided urine sufficiently concentrated in drug content for the analyses of minor metabolites.

GC and GC/MS Quantitation. Standard curves for all compounds were prepared by adding known amounts (0–40 μg) as the internal standard. The samples were extracted with or without acid-hydroysis and analyzed by GC. For GC/MS analyses, the samples were derivatized with Tri-Sil Z before injection. Standard curves were constructed by plotting peak height ratios *vs.* concentration. Linear relationships for all components were observed for this concentration range. Prediction limits were constructed from these data, which were used to monitor the day-to-day variability of standard control samples, thus providing a quantitative measure of the reliability of the standard curve. All samples were analyzed in triplicate and the means are reported.

Results

Analysis of the urine samples collected from man after a single 15-mg oral dose of HC revealed a complex pattern of metabolism as shown in fig. 1. The routes of metabolism included O-demethylation, N-demethylation, and 6-keto reduction to the corresponding 6-α- and 6-β-hydroxy metabolites. The identity of all drugs shown in fig. 1 was confirmed by comparison of their retention times and mass spectral patterns (CI-methane) with authentic standards. Untreated and acid-hydrolyzed urine samples from six subjects were analyzed by GC for HC, 6αHC, 6βHC, and NC content (fig. 2). In addition, the acid-hydrolyzed urine samples for two subjects were analyzed by GC/MS for HC, 6αHC, 6βHC, HM, 6αHM, and 6βHM. The combined results from these studies are shown in table 2. Similar analyses of urine samples from dog, rat, guinea pig, and rabbit are reported in table 3.

TABLE 1

Summary of protocol for animal experiments

Drug was administered po to human subjects and sc to the other species.

Species	N	Average Weight	Dose of HC
		kg	*mg*
Man	6	83.2	15
Rat	6	0.46	5
Dog	2	8.2	10
Guinea pig	6	1.02	5
Rabbit	4	4.40	5

Approximately 50% of the total drug recovered from urine of man was HC which represented *ca.* 11% of the administered dose. The remaining half was divided among the metabolites in the following order of decreasing abundance NC > HM > 6βHC ≃ 6αHC > 6βHM > 6αHM. HC, NC, and HM were detectable through 72 hr, whereas the 6-hydroxy metabolites were generally detectable for shorter periods of time. Analyses of acid-hydrolyzed samples showed only slight increases in the amount of NC, 6αHC, and 6βHC in urine, indicating that conjugation was minimal for HC and metabolites in man.

Individual variation in the amounts of HC and metabolites excreted in the urine of man was considerable. The total cumulative excretion of HC ranged from 6–20% of the administered dose for the six subjects. The individual totals for NC ranged from 2–14%. Excretion totals of 6αHC and 6βHC were quite similar and ranged from 1–3%. It was noted that the individual who excreted the most HC (subject 6) also excreted the largest amount of metabolites. The excretion pattern for subject 5 was similar to that of subject 6, whereas the excretion data of the remaining four subjects were clustered much closer together. Subjects 5 and 6 were the youngest of the group and also weighed the least; however, their differences from the remainder of the group hardly seem enough to explain the apparent clustering of the subjects into two groups. Further work is needed to establish the significance of this pattern.

The patterns of metabolism of HC by other species in comparison to man are shown in table 3. Recovery of HC was relatively low and ranged from about 1 to 4% of the administered dose. The 6-α- and 6-β-hydroxy metabolites of HC and HM were detected in the urine of rat and guinea pig whereas only the alcoholic metabolites of HM

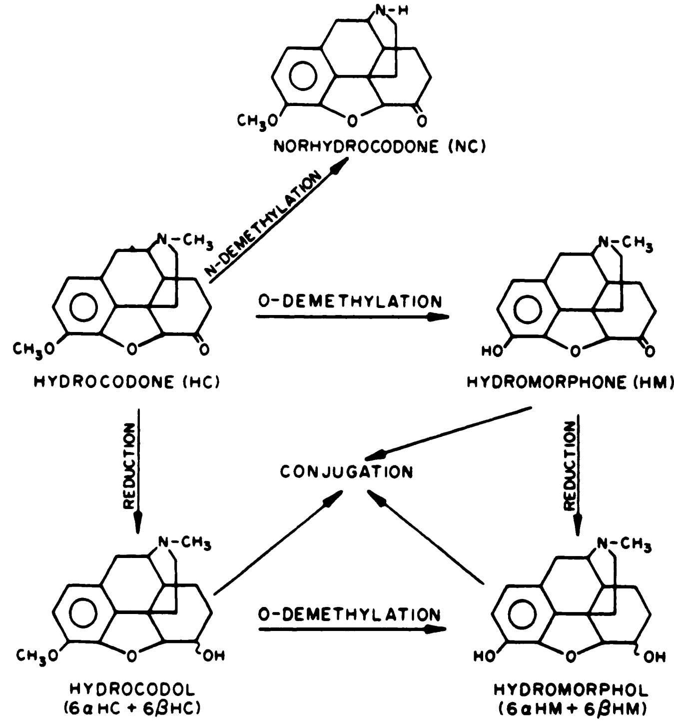

FIG. 1. *Structures of hydrocodone and metabolites identified in urine of man.*

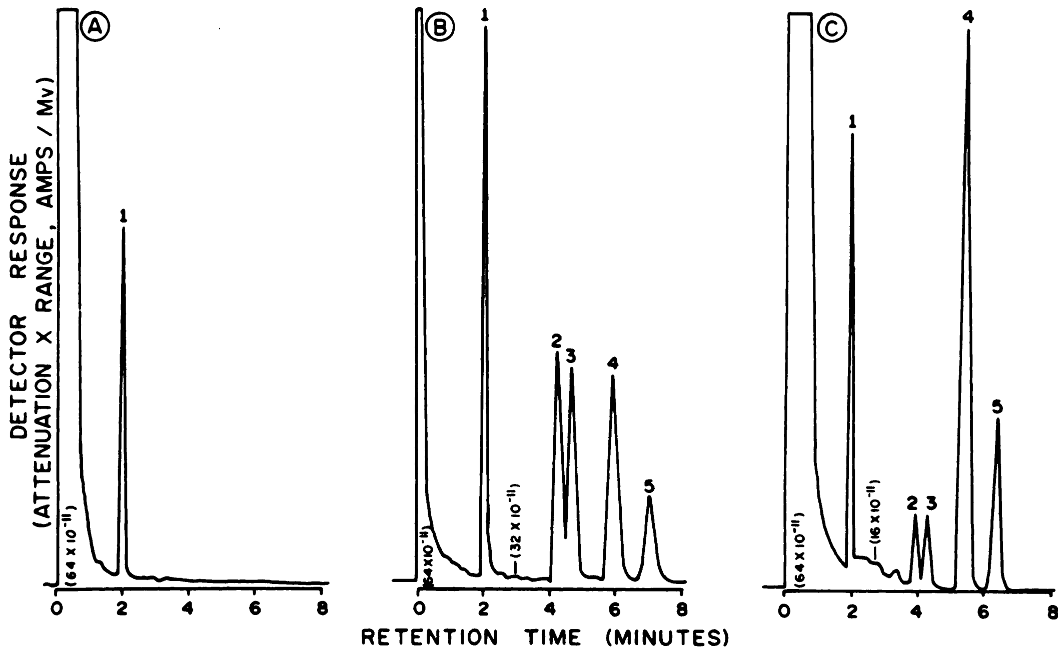

FIG. 2. *Gas chromatogram of human urinary extracts.*

A, Drug-free urine with added internal standard, cyclazocine (*1*); *B*, drug-free urine with added *1*, 6αHC (*2*), 6βHC (*3*), HC (*4*), and NC (*5*); *C*, urine collected 2–4 hr after drug administration with added *1*.

TABLE 2

Mean urinary excretion of hydrocodone and metabolites from six human subjects

Values represent means of triplicate determinations and are expressed as percent of administered dose. *T* denotes trace amount of drug detected (<0.1% of the administered dose).

Drug	Sample Treatment[a]	Excretion During Collection Period (hr) of							
		0–2	2–4	4–8	8–12	12–24	24–48	48–72	Total[b]
HC	—	2.3	1.8	1.8	0.5	2.2	2.0	1.0	11.6 (2.4)
	AH	2.1	1.9	1.6	0.6	1.5	2.0	1.3	11.0 (1.2)
6αHC	—	0.1	0.1	0.2	0.1	0.5	0.6	0	1.6 (0.3)
	AH	0.1	0.2	0.3	0.1	0.5	0.6	0	1.8 (0.3)
6βHC	—	0.1	0.1	0.2	0.1	0.5	0.6	0	1.6 (0.4)
	AH	0.2	0.3	0.3	0.1	0.8	0.5	0	2.2 (0.6)
NC	—	0.6	0.5	0.7	0.3	1.6	1.2	0.3	5.2 (1.8)
	AH	1.2	1.2	1.1	0.4	1.2	1.0	0.4	6.5 (1.0)
HM[c]	AH	0.4	0.6	0.2	0.5	0.7	0.8	0.3	3.5 (0.4)
6αHM[c]	AH	T	T	T	T	T	0	0	<0.1 —
6βHM[c]	AH	T	0.1	T	T	T	0	0	0.1 (0)

[a] Samples were analyzed for drug and metabolite content with (AH) and without (—) acid-hydrolysis.
[b] Data in parentheses are the standard errors of the means between subjects.
[c] These data represent the means obtained from analyses by GC/MS for two subjects.

were detected for the rabbit. No reduction products were detected for the dog. HM was detected in the urine of all species. Of the four laboratory animals species, the N-dealkylated metabolite, NC, was detected only in the urine of the dog. Percent conjugation was highest for HM and 6βHM in the rabbit and NC in the dog. Overall recoveries of drug and metabolite ranged from a low of 10.6% for the rabbit to a high of 46.8% for the guinea pig with rat, dog, and man falling in the midrange.

Discussion

The metabolism of HC in man and animals includes several biotransformation pathways common to opiate agonists and antagonists. These include N-demethylation of heroin (9), morphine (10), and codeine (11), O-demethylation of codeine (12), and 6-ketoreduction of naltrexone (3), naloxone (4, 5), and hydromorphone (6). As applied to HC, each type of metabolic transformation presumably results in the production of an active metabolite. Although the pharmacological activity of NC is not known, it is likely that NC exhibits some analgesic activity because by analogy to codeine, norcodeine is active (13). The analgesic activity of the remaining metabolites of HC as well as that of HC and morphine have been reported by Small *et al.* (15). The relative analgesic potencies as compared to morphine are as follows: HC, 0.59; 6αHC, 0.10; 6βHC, 0.40; HM, 4.40; 6αHM, 2.90; 6βHM, 0.94. All of these metabolites display activity approximately equal to or greater than that of the parent compound, with the exception of 6αHC which is about ⅙ as potent.

The analgesic activities of the O-demethylated metabolites are significantly greater (2 to 7-fold) than that of HC or the reduced metabolites, 6αHC and 6βHC. Consequently, the ratio of O-demethylated metabolites to parent drug excreted in urine could possibly be a rough indicator of the relative contribution of activity of those metabolites to the overall pharmacology of HC. The ratio (HC + 6αHC + 6βHC)/(HM + 6αHM + 6βHM) for each species might be useful in this regard. Combination of data from table 3 provides these ratios for each species as follows: man, 4.3; rat, 1.0; guinea pig, 4.9; dog, 2.6; rabbit, 0.1. On this basis, the relative importance of the contribution of activity by O-demethylated metabolites should be in descending order: rabbit > rat > dog > man ≃ guinea pig. Numerous metabolic processes might intervene to limit the usefulness of these ratios, including the degree of conjugation of the O-demethylated metabolites and possibly their recycling by enterohepatic recirculation. In addition, a large proportion of the administered dose could not be accounted for in some species. However, it seems likely that O-demethylation of HC and metabolites may be an important factor in the development of analgesia for some species, most notably in the rabbit and rat.

Considerable species differences in the metabolism of HC were observed as was the case for the

TABLE 3

Recovery of drug and metabolites from urine of animals administered hydrocodone

Values represent means of triplicate determinations and are expressed as percent of administered dose. Levels of conjugated (*conj.*) drug and metabolites were determined by subtraction of free from total concentration after acid-hydrolysis

Speices	HC		6αHC		6βHC		NC		HM		6αHM		6βHM		Total Free	Total Conj.	Total Drug
	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.			
Man	11.6	0	1.6	0.2	1.6	0.6	5.2	1.3	3.5[a]		<0.1[a]		0.1[a]				25.7
Rat	3.6	0	0.1	0	4.5	0	0	0	0.8	0.6	0.1	0	2.1	4.8	11.2	5.4	16.6
Guinea Pig	3.6	0	2.4	0	32.9	0	0	0	3.4	0	0.6	0	3.9	0	46.8	0	46.8
Dog	3.6	0	0	0	0	0	15.0	4.5	0.2	1.2	0	0	0	0	18.8	5.7	24.5
Rabbit	0.7	0	0	0	0	0	0	0	0.6	6.0	0	0.4	0.1	2.8	1.4	9.2	10.6

[a] These data represent the means from two subjects and were analyzed only for total concentration after acid-hydrolysis.

metabolism of HM (6). The predominant urinary metabolite was different in each species (table 3). Also, in contrast to the metabolism of HM, considerable amounts of the N-demethylated metabolite, NC, were found for man and dog. This parallels the differences found for codeine and morphine where codeine is N-dealkylated to a greater extent than morphine in man (10, 14). It is possible that these differences in N-demethylation are due to the greater lipophilic nature of HC *vs.* HM, which may provide greater accessibility of HC to the N-demethylating system. In addition, HM has a phenolic site suitable for conjugation which may provide an alternate metabolic pathway for elimination.

Considerable stereoselectivity was observed for the 6-keto reduction of HM in man and animals in a previous study (6). A similar observation was made in this study for the reduction of HM which was produced *in vivo* by O-demethylation of HC. Stereoselectivity also was observed for the reduction of HC in the laboratory animal species. However, there was a surprising lack of selectivity in the formation of 6αHC and 6βHC from HC in man. A possible explanation of these differences in stereoselectivity of reduction might be that different enzymes are involved in the reduction of HC *vs.* HM. Differences in the distribution of the substrates might lead to the observed differences in stereoselectivity. The lack of stereoselectivity of HC in man, however, remains a puzzle.

In man the time course of excretion of HC and metabolites was similar to that of HM (6). Generally more than 70% of the total excretion of HC or metabolite occurred in the first 24 hr. The reduced metabolites were generally detectable only through 24–48 hr, whereas HC, NC, and HM were detectable through 72 hr. There was considerable individual variation in the amounts of drug and metabolite excreted by man. Overall recoveries of HC seemed to be clustered into two groups; four subjects excreted 6–9% and two excreted 18–20% HC (as percentage of the administered dose). A similar pattern was observed in the excretion of 6αHC and 6βHC; the same two subjects excreted considerably higher amounts than the remaining subjects.

Overall recoveries of HC and metabolites were rather low, being in the range of 11–47% of the administered dose. This is in contrast to the considerably higher recoveries found for HM and metabolites for the same species (29–73% of the administered dose) (6). The remainder of the dose of HC was not accounted for. Alternate pathways

of elimination as well as other modes of metabolism are likely explanr.tions.

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