J. Pharm. Pharmacol. 1979, 31: 314–317 Received July 11, 1978

Comparative metabolism of codeine in man, rat, dog, guinea-pig and rabbit: identification of four new metabolites

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The metabolism and excretion of codeine and its metabolites in untreated urine of man, rat, dog, guinea-pig and rabbit have been examined. Metabolites were identified by gas chromatography mass spectrometry operated in the chemical ionization mode (methane). Concentrations of codeine and metabolites were measured by selected ion monitoring. Both codeine and norcodeine were detected in the urine of all species but a new metabolite, hydrocodone, was found only in the urine from man, guinea-pig and dog. Additional metabolites (presumably resulting from the metabolism of hydrocodone) were also detected in man and guinea-pig. Overall recoveries of drug and metabolites from untreated urine were low for all species.

For some time our laboratory has been engaged in metabolism studies on narcotic agonists and antagonists in an attempt to define general patterns of species differences in the metabolism and excretion of analgesics. We report here a study of the excretion of codeine, norcodeine and four new metabolites in untreated urine of man, rat, dog, guinea-pig and rabbit following codeine administration.

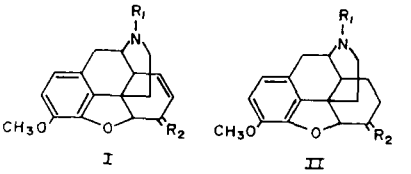
MATERIALS AND METHODS

Compounds. Codeine (Ia) and potential metabolites (Ib, Ic, IIa–IId) were available as authentic reference standards. Cyclazocine was used for internal standardization. All compounds were checked for purity by t.l.c., g.l.c. and mass spectrometry.

Animals. One female and one male mongrel beagle (7·2 and 9·3 kg), 7 male rats (albino Wistar, 410–480 g), 4 male rabbits (albino New Zealand, 3·3–3·6 kg) and 5 male guinea-pigs (albino Hartley, 750–1050 kg) were used.

Human study. Two healthy adult male volunteers (both 75·0 kg), who gave their informed consent, participated.

Drug administration and urine collection. Animals were housed in metal cages with stainless steel urine collection pans. Control urine was collected the day before drug administration. A single dose of codeine was administered subcutaneously to dogs (10 mg), rats (5 mg), rabbits (5 mg), and guinea-pigs (5 mg) and the urine was collected for the next 24 h. Then the cages were rinsed with water, which was added to the urine. Faeces which were separated from the urine were discarded.



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Compound	R_1	R_2	Name
Ia	CH_3	H, OH	Codeine
Ib	CH_3	OH, H	α-Isocodeine
Ic	H	H, OH	Norcodeine
IIa	CH_3	O	Hydrocodone
IIb	CH_3	H, OH	6α-Hydrocodol
IIc	CH_3	OH, H	6β-Hydrocodol
IId	H	O	Norhydrocodone

FIG. 1. Structures of metabolic products of codeine (Ia). α-Isocodeine (Ib) is included but was not detected as a metabolite.

The volunteers were drug-free when control urine was collected. After one dose of codeine (30 mg by mouth) urine was collected for 24 h, the urine samples being divided into two 12 h pools for each subject.

All urine was frozen until analysis.

Gas-liquid chromatography. A Varian Model 2700 gas chromatograph, equipped with a flame ionization detector, was used for extraction and recovery studies.

G.l.c.: 1·82 m × 2 mm glass column packed with 3% Silar 5CP on Gas-Chrom Q (100/120 mesh); carrier gas: nitrogen 21 lb in^{-2} (483 kN m^{-2}) (50 cm^3 min^{-1}); column temperature, 250 °C; injector and detector temperatures, 275 °C.

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Gas chromatography-mass spectrometry. The mass spectra of codeine and its metabolites and the internal standard, cyclazocine, were obtained on a Finnigan Model 3300 quadrupole g.c.-mass spectrometer operating in the chemical ionization mode and equipped with a Finnigan Model 6000 Interactive Data System. G.l.c. system: glass column (1·52 m × 2 mm) packed with 3% Silar 5CP on Gas-Chrom Q (100/120 mesh) coupled by a glass lined stainless steel tube to the mass spectrometry. Electron energy was 80 eV. Carrier and reagent gas: methane with a flow rate giving an ion source pressure of 0·133 kN m^{-2} (1 Torr) was used. Temperatures of the injectors, column and ion source: 270, 250 and 100 °C, respectively.

After injection of the sample the venting valve was opened for 20 s to allow solvent and highly volative substances to escape without entering the ion source.

Extraction efficiency study. Control urine samples containing codeine and metabolites were extracted as described using n-butyl chloride or chloroform with isopropanol (0–20% v/v). Percent recoveries of drugs were calculated relative to the same amounts of unextracted standards.

Extraction of samples. Urine samples (10–20 ml) were saturated with sodium chloride, buffered to pH 10 ± 0·1 with K_2HPO_4 (40%) and extracted with equal volumes of chloroform. Cyclazocine (50 μg) was added before extraction for internal standardization. The organic layer was transferred to a tube containing 3 ml of 2M HCl. After it had been shaken (5 min), the organic layer was removed and the pH of the aqueous phase adjusted to pH 10 ± 0·1 with sodium hydroxide and buffer. The aqueous phase was saturated with sodium chloride and extracted with 15 ml of chloroform. The organic phase was removed, evaporated, and the residue was taken up in methanol (50 μl) for analysis.

Stability of codeine (Ia) and hydrocodone (IIa) in urine. Control urine with either Ia or IIa added (100 μg) was incubated at room temperature (70 °C) for 2 days. Internal standard was added and the samples were extracted in the usual manner and analysed.

Quantitative analysis. Standard solutions of Ia and metabolites in control urine containing internal standard (50 μg/10 ml) were prepared as follows: Ia (0–40 μg/10 ml); Ib, Ic, IIa–IId (0–10 μg/10 ml). These solutions were extracted as described and analysed by g.l.c.-m.s. (in the methane chemical ionization mode). Selected ion recording was used for quantitative analysis. The ions found to be specific for each compound at its respective retention time were as follows: Ia, *m/e* 302 (6·45 min); Ib, *m/e* 302 (9·05 min); Ic, *m/e* 268 (10·2 min); IIa, *m/e* 300 (11·5 min); IIb, *m/e* 302 (5·70 min); IIc, *m/e* 302 (7·40 min), IId, *m/e* 286 (17·10 min); cyclazocine, *m/e* 300 (2·80 min). Plots of peak height ratios of compound/internal standard versus concentrations were linear throughout the concentration range tested. The lower limits of detection of all compounds by this method was approximately 10 ng ml^{-1}. Urine samples collected after drug administration with internal standard added (50 μg) were similarly analysed.

A daily standard curve was constructed from a set of 5 concentration points for all compounds. Least squares linear regression of these data provided slopes and intercepts for calculation of drug content of samples.

RESULTS AND DISCUSSION

Extractability and stability of metabolites of codeine. Of the several solvent systems tested, chloroform alone gave the best overall results with recoveries in the range of 42–63%. As the polarity of the organic solvent was increased by adding increasing amounts of isopropanol, recoveries decreased. n-Butyl chloride gave the lowest recoveries in most cases.

Both codeine and hydrocodone were unaffected in the stability study; this rules out the possibility of artifact formation.

Chromatographic and mass spectral characteristics of codeine and metabolites. Several stationary liquid phases (e.g. Silar 5 CP, Silar 10 CP, OV-17, OV-225) were tested for their ability to separate Ia and metabolites. Silar 5 CP (3%) gave the best overall results. Table 1 contains the uncorrected retention times and relative retention times of Ia and metabolites on Silar 5 CP at 250°C. Near baseline resolution was observed for all components with the exception of Ic and IIa which were only approximately 50% resolved.

The methane chemical ionization spectra of codeine and its metabolites and cyclazocine were obtained by g.c.-m.s. (Table 1). With the exception of codeine and Ic the most abundant ion for each compound was the protonated molecular ion, $(M + 1)^+$. The most abundant ion of both codeine and Ic was the $(M-17)^+$ ion resulting from loss of water from the protonated molecular ion.

Identification of four new metabolites (IIa–IId) of codeine in man and guinea-pig urine. Four new metabolites of codeine were detected and identified in untreated human and guinea-pig urine as hydrocodone (IIa), 6α-hydrocodol (IIb), 6β-hydrocodol (IIc), and

Table 1. G.l.c. and methane chemical ionization (CI) spectra of codeine and metabolites.

Compound	G.l.c. Rt, min[a] (Rel. R_F)		Mol. wt	Methane CI spectra[b] $(M+29)^+$	$(M+1)^+$	M^+	Prominent fragment ions
Ia	6·45	(2·30)	299	328 (8)	300 (28)	299 (23)	298 (10), 283 (19), 282 (100), 262 (12)
Ib	9·05	(3·23)	299	328 (14)	300 (100)	299 (45)	298 (22), 283 (13), 282 (71), 262 (20)
Ic	10·20	(3·64)	285	314 (8)	286 (34)	285 (22)	287 (5), 284 (7), 269 (17), 268 (100), 262 (27)
IIa	11·50	(4·11)	299	328 (10)	300 (100)	299 (23)	301 (21), 262 (13), 257 (31)
IIb	5·70	(2·04)	301	330 (13)	302 (100)	301 (33)	303 (20), 300 (17), 284 (15)
IIc	7·40	(2·64)	301	330 (14)	302 (100)	301 (41)	303 (19), 300 (23), 284 (43)
IId	17·10	(6·11)	285	314 (8)	286 (100)	285 (20)	287 (16), 284 (6), 262 (36)
Cyclazocine	2·80	(1·00)	271	300 (11)	272 (100)	271 (52)	273 (19), 270 (25), 262 (17)

[a] G.l.c. conditions were as follows: 2 mm × 1·82 m glass column packed with 3% Silar 5CP on Gas-Chrom Q (100/120 mesh). Nitrogen carrier flow, 50 cm^3 min^{-1}; column temperature, 250°C; injector and detector temperature, 275°C.

[b] *m/e* (percent abundance).

norhydrocodone (IId). The metabolites were identified by comparison of their g.l.c. and mass spectral characteristics with those of authentic standards (Fig. 2). Ib was not detected in the urine of any species.

Three of the four new metabolites exhibit greater analgesic activity than codeine (Small et al 1938). The activity of IId has not been reported. Although the amounts of IIa–IId detected in the urine of man and guinea-pig were small, their contribution to the pharmacological activity of codeine remains a possibility.

The biotransformation of codeine to IIa is analogous to that reported for the conversion of morphine to hydromorphine in rat (Klutch 1974; Yeh et al 1977) and mouse, cat, rabbit, guinea-pig and monkey (Yeh et al 1977). However, this is the first report of this type of biotransformation of a narcotic analgesic in man.

It is likely that IIb, IIc, and IId are products of metabolism of IIa since these compounds have been identified in the urine of human subjects who had ingested IIa (Cone et al 1978).

Quantitative analysis of Ia and metabolites. Selected ion recordings of masses at *m/e* 268, 286, 300 and 302 by g.c.-m.s. (methane chemical ionization) using a 3% Silar 5 CP liquid coating for g.c. provided a sensitive and specific mode of analysis of codeine, Ib, Ic and IIa—IId in urine. Daily calibration curves of peak height ratios of compound/internal standard were linear (correlation coefficients of 0·99–1·00). Typical recordings of urine extracts of standards, controls and drug samples are shown in Fig. 2.

Species differences. Significant species differences were observed in the excretion pattern of free codeine and metabolites in the untreated urine of all species following codeine administration (Table 2). The

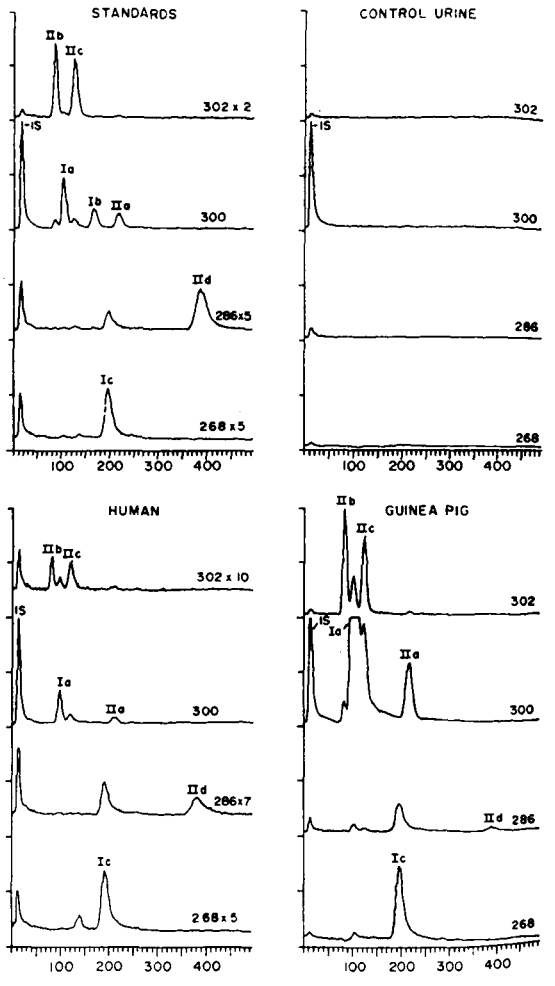

FIG. 2. Selected ion recordings of standards, control urine and codeine urine extracts to which 50 μg of internal standard (cyclazocine) was added. Ordinate: detector response. Abscissa: scan number.

Table 2. Mean 24 h excretion of codeine and metabolites from untreated urine[a].

Species	Time (h)	Route of adm.[b]	Ia	Ic	IIa	IIb	IIc	IId	Total
Human									
Subject 1	0–12	p.o.	5·56	3·83	0·02	0	0·02	0·06	9·49
	12–24		0·46	1·10	0·03	0·02	0·02	0·06	1·69
Subject 2	0–12	p.o.	5·00	0·96	0·04	0	0	0·05	6·05
	12–24		0·43	0·13	0·06	0·03	0·04	0·07	0·76
Dog	0–24	s.c.	2·86	9·47	0·01	0	0	0·04	12·38
Guinea-pig	0–24	s.c.	3·78	0·17	0·10	0·08	0·08	0·02	4·23
Rabbit	0–24	s.c.	0·10	0·10	0	0	0	0	0·20
Rat	0–24	s.c.	6·50	0·23	0	0	0	0	6·73

[a] Mean of duplicate or triplicate analyses expressed as percent administered dose.
[b] The abbreviations p.o. and s.c. represent oral and subcutaneous drug administration.

most abundant metabolite measured in the urine was the *N*-dealkylated product Ic. The recoveries of Ic ranged from a low of 0·10% for rabbit to a high of 9·47% for dog. Smaller amounts of IIa were found in the urine of man, dog, and guinea-pig. Other metabolites detected which presumably resulted from the metabolism of IIa included IId in the urine of man, guinea-pig and dog, and IIb and IIc in the urine of man and guinea-pig. The presence of IId and the absence of IIb and IIc in dog urine are consistent with the report of the lack of reduction of IIa in the dog (Cone et al 1978).

Overall recoveries of codeine and metabolites from untreated urine were low, ranging from 0·2% for the rabbit to a high of 12·4% for the dog. This is consistent with other reports on the metabolism of codeine in various species in which large amounts of codeine and metabolites were found to be excreted in the conjugated form (Way & Adler 1960; Yeh & Woods 1971; Yoshimura et al 1970) and presumably account for the remainder of the dose.

Acknowledgements

The authors thank Mr W. G. Marquardt and Ms M. L. Scouten for expert technical assistance. We also thank Dr J. W. Barnhart, Dow Chemical Co., for a generous gift of IId.

REFERENCES

Cone, E. J., Darwin, W. D., Gorodetzky, C. W., Tan, T. (1978) Drug Metab. Dispos. 6: 488–493

Klutch, A. (1974) Ibid 2: 23–30

Small, L. F., Eddy, N. B., Mosettig, E., Himmelsbach, C. K. (1938) 'Studies on Drug Addiction,' Public Health Reports Suppl. 138, U.S. Government Printing Office, Washington, D.C.

Way, E. L., Adler, T. K. (1960) Pharmacol. Rev. 12: 383–446, and references therein

Yeh, S. Y., McQuinn, R. L., Gorodetzky, C. W. (1977) Drug Metab. Dispos. 5: 335–342

Yeh, S. Y., Woods, L. A. (1971). Arch. Int. Pharmacodyn. Ther. 181: 231–242

Yoshimura, H., Mori, M., Oguri, K., Tsukamoto, H. (1970) Biochem. Pharmacol. 19: 2353–2360