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METABOLISM *IN VITRO* AND *IN VIVO* OF PENTAZOCINE

K. A. PITTMAN, D. ROSI, R. CHERNIAK,* A. J. MEROLA† and W. D. CONWAY‡

Sterling–Winthrop Research Institute, Rensselaer, N.Y. 12144, U.S.A.

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Abstract—The metabolism of pentazocine has been investigated in the 10,000 *g* supernatant of monkey, mouse and rat liver homogenates and in the monkey, *in vivo*. Extracts of the incubations *in vitro* and of monkey urine were examined for pentazocine and possible metabolites. Tritium-labeled pentazocine was used to quantitate the excretion of the drug after administration and to measure the recovery of metabolites. The results indicate that the metabolism of pentazocine occurs mainly through oxidation of the terminal methyl groups of the dimethylallyl side-chain to yield two isomeric alcohols and one of the corresponding carboxylic acids. Some of the pentazocine and its metabolites were present in the urine as unidentified conjugates. About 70 per cent of the radioactivity administered as pentazocine was recovered in 24 hr in the urine of monkeys receiving widely varying doses of the drug.

PENTAZOCINE, a benzomorphan derivative, is a potent, non-narcotic analgesic.[1,2] A parenteral form of the drug is currently available and knowledge of its metabolism is, therefore, increasingly important in order to conduct studies of the effect of dosage form and route of administration upon the action of the drug in man. Studies of blood levels of the drug in human subjects after intravenous, intramuscular and oral administration have been reported.[3,4] These have shown that the drug has a plasma half-life of about 2–2·5 hr in man and that it is capable of passing the placental barrier.[4] The appearance of radioactivity in the urine of patients administered tritium-labeled pentazocine accounted for about 50 per cent of the dose in 12 hr.[5] The following studies were carried out to provide basic information concerning the chemical identity of the biotransformation products of pentazocine produced in animals, as a prelude to further studies of the drug in man.

MATERIALS AND METHODS

Pentazocine [1,2,3,4,5,6-hexahydro-*cis*-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocine-8-ol, Fig. 1, a.] and related compounds were prepared by Dr. N. F. Albertson, and pentazocine labeled with tritium in the 4-position was prepared by Dr. G. D. Diana, both of Sterling–Winthrop Research Institute. The

* Present address: Department of Chemistry, Georgia State College, Atlanta, Ga., U.S.A.
† Present address: Department of Physiological Chemistry, Ohio State University, Columbus, O., U.S.A.
‡ Present address: Department of Pharmaceutics, State University of New York, Buffalo, N.Y., U.S.A.

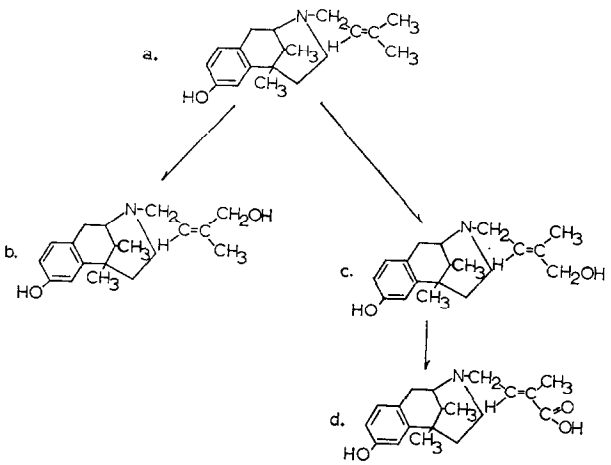

FIG. 1. The biotransformation of pentazocine in the intact rhesus monkey and in the 10,000 *g* super natant fractions of livers obtained from the rat, mouse and rhesus monkey.

purity of all compounds was determined by thin-layer and gas–liquid chromatography. Radioactive preparations were always assayed just prior to use.

Studies in vitro. Livers were taken from 3-kg rhesus monkeys of either sex, 20–25 g Swiss–Webster mice of either sex and 150–200 g male Sprague–Dawley rats, and were immediately washed in ice-cold 1·15% KCl. Weighed liver samples were homogenized in 3 vols. of ice-cold 1·15% KCl, centrifuged at 10,000 *g* and the resulting supernatant was used directly as a source of microsomes and an NADPH-generating system. The incubation mixtures were composed of the following: 20% (v/v) of the 10,000 g supernatant described above; 0·05 M phosphate buffer, pH 7·6; 2×10^{-3} M nicotinamide; 1×10^{-4} M NADP; $5{\cdot}6 \times 10^{-3}$ M glucose-6-phosphate; 1×10^{-3} M $MgSO_4$; 2×10^{-4} M EDTA and 2×10^{-3} M pentazocine. Incubations were carried out under air at 37° in a shaking water bath for 1 hr and stopped either by adjusting to pH 10 with 10% NaOH and extracting with ethyl acetate or by adding 10 vols. of ethanol and centrifuging. Purification and analysis of extracts were performed as described later.

Studies in vivo. Female rhesus monkeys received aqueous pentazocine lactate i.m., with no more than 50 mg being given in any 2-hr period. The monkeys were restrained in a primate chair; their urine was collected separately from feces and maintained at 0° in the presence of a little toluene. At the end of each collection period, 400 ml of 0·1 N acetate buffer, pH 5·5; 0·25 ml glusulase (containing approximately 150,000 units of β-glucuronidase and 40,000 units of phenol sulfatase per ml, Endo Research Labs., Inc.) and 1 ml of chloroform were added per 100 ml of urine and the preparation was incubated overnight at 37° under a nitrogen atmosphere. The diluted urine solution was then reduced to $\frac{1}{10}$ the original urine volume, absolute ethanol to twice the original volume added and the solution was allowed to stand overnight in the cold before decanting it and washing the precipitate twice with small volumes of ethanol. The combined ethanolic solutions were taken to dryness under a stream of

air and the residue taken up in water and passed through a 100–200 mesh Dowex 50W-X8 column in the hydrogen-ion form. The column was rinsed with water until neutral and pentazocine and its metabolites were eluted with 2 N ammonia in 60% methanol. The eluate was taken to dryness, the residue extracted several times with small volumes of methanol and the extract used for further analysis.

Analysis of extracts. Thin-layer chromatography was performed in one and two dimensions in the solvent systems described in Table 1 on 250 μ layers of silica gel G

TABLE 1. RELATIVE R_f VALUES OF COMPOUNDS PRESENT AFTER INCUBATIONS *IN VITRO* OF PENTAZOCINE WITH 10,000 *g* SUPERNATANTS OF MONKEY, MOUSE AND RAT LIVER HOMOGENATES AND PRESENT IN THE URINE OF MONKEYS ADMINISTERED PENTAZOCINE*

	Solvent system†				
Compound‡	1	2	3	4	5
A	1·00	1·00	1·00	1·00	1·00
B	0·73	0·85	0·96	0·72	0·54
C	0·44	0·61	0·92	0·63	0·35
D	0·05	0·51	0·23	0·82	0·00

* R_f values are relative to the R_f of authentic pentazocine. The absolute R_f values of authentic pentazocine in these solvent systems were as follows: 0·65(1), 0·45(2), 0·87(3), 0·70(4), and 0·57(5)

† The solvent systems were prepared by volume as follows: 1. benzene–methanol–isopropylamine, 95:5:3; 2. dioxane–water–acetic acid, 100:2:5; 3. dioxane–water–ammonia, 100:15:5; 4. dioxane–methanol–formic acid, 100:5:5; 5. ethyl acetate–triethylamine, 90:10.

‡ For the structural formulae of these compounds see Fig. 1.

(E. Merck, A. G., Darmstadt) and on commercially prepared silica gel plates containing a fluorescent indicator (E. Merck, A. G., Darmstadt). Spots were visualized with short-wave ultraviolet light, iodine vapor or Dragendorff reagent. Components of interest were eluted from the silica gel with methanol.

Gas–liquid chromatography was performed using an F and M model 402 gas chromatograph with a flame ionization detector and helium carrier gas at temperatures between 230° and 250° on four-foot glass columns packed with 3% OV-1 and OV-17 on Gas Chrom Q (Applied Science Labs.) Trimethylsilyl-derivatives were prepared with DMF Sil-Prep (Applied Science Labs., Inc.).

Radioactivity was measured in a Packard model 3003 Tri-Carb scintillation spectrometer in a dioxane based scintillation solution,[6] using the channels-ratio method of quench correction.[7] Radioactivity on thin-layer plates was measured by scraping the gel into vials, adding scintillator and counting directly. There were no differences in the corrected count rates of solutions determined directly or after drying on thin-layer plates. Radioactivity on narrow plates was detected in a Packard radio-chromatogram scanner model 7201, and on wide plates by scraping and counting.

Infra-red spectra were run on a Beckman model IR7 infra-red spectrophotometer and n.m.r. spectra on a Varian model A-60 nuclear magnetic resonance spectrometer.

RESULTS AND DISCUSSION

Metabolism in vitro. Ethyl acetate extracts of incubation mixtures of pentazocine with monkey, mouse and rat 10,000 *g* liver supernatant fractions revealed the presence of unaltered drug (A) and two more polar compounds (B, C). On occasion, ethanolic extracts of the monkey liver preparations contained an additional compound (D) which was much more polar than the parent drug or the other 2 metabolites. The mouse liver preparation produced five to ten times as much of metabolite C as of metabolite B. The rat liver preparation produced two to three times as much of B as of C, while the monkey liver preparation produced approximately equal amounts of these 2 metabolites. The relative R_f values and retention times of these metabolites as compared to those of authentic pentazocine are listed in Tables 1 and 2.

TABLE 2. RELATIVE RETENTION TIMES OF COMPOUNDS PRESENT AFTER INCUBATIONS *IN VITRO* OF PENTAZOCINE WITH THE 10,000 *g* SUPERNATANTS OF MONKEY, MOUSE AND RAT LIVER HOMOGENATES AND PRESENT IN THE URINE OF MONKEYS ADMINISTERED PENTAZOCINE*

Compound	Form studied			
	Free†	Trimethylsilyl derivative†	Free‡	Trimethylsilyl derivative‡
A	1·00	1·00	1·26	1·26
B	2·06	2·22	2·60	2·80
C	2·30	2·70	2·90	3·40
D		3·65		4·60

* Values were determined on 4′, 3 per cent OV-1 columns on Gas Chrom Q.
† Retention times are relative to that of authentic pentazocine.
‡ Retention times are relative to that of docosane.

TABLE 3. CUMULATIVE PER CENT OF DOSE APPEARING IN THE URINE OF MONKEYS GIVEN TRITIUM-LABELED PENTAZOCINE INTRAMUSCULARLY

Monkey	Dose (mg/kg)	Hours after administration								
		1	2·5	3·5	9	20	24	48	72	96
N	0·3	0	29·4	42·9	52·6	65·3	67·3	67·3		
Q	34·1						60·8	72·9	76·0	
S	15·7						69·1	74·0	76·3	78·0
T	0·3				50·9		62·3	66·0	67·2	

Metabolism in vivo. The appearance of tritium in the urine of four monkeys receiving an intramuscular dose of labeled pentazocine was rapid (Table 3), with about 70 per cent of doses ranging from 0·3 to 34 mg per kg being excreted within 24–48 hr after administration. The reported excretion in 48 hr of 65 to 75 per cent of the tritium from labeled pentazocine given to human subjects[5] is similar to the results obtained with monkeys in this study. The amount of radioactivity appearing in the feces of monkey S after 96 hr was less than 3 per cent of the dose, and in that of monkey T after 56 hr was 6 per cent of the dose.

The method used for the extraction and preliminary purification of metabolites from hydrolyzed urine allowed the recovery in the final methanol extract of 95 per cent of the radioactivity of a given urine sample. Extraction with less polar solvents, such as acetone, or by methods similar to those used by Mulé[8] resulted in large and variable losses, because pentazocine and the less polar metabolites were only partially extracted and the more polar metabolite was extracted not at all.

After hydrolysis, the urine of each of seven monkeys yielded the same four compounds as were recovered from the studies *in vitro*. The relative amounts found in three monkeys administered tritium-labeled pentazocine are shown in Table 4. The

TABLE 4. DISTRIBUTION AMONG SEVERAL COMPOUNDS OF THE RADIOACTIVITY IN THE 24-hr URINE OF MONKEYS ADMINISTERED ^{3}H-PENTAZOCINE*

Compound†	Per cent of radioactivity in 24-hour urine		
	Monkey N	Monkey S	Monkey T
A	29·7	20·6	30·2
B	16·3	19·0	21·0
C	15·9	13·0	7·4
D	13·2	36·2	34·9
Total	75·1	88·8	93·5

* Urines were treated with glusulase (Endo Labs, Inc.) before examination.

† For the structural formulae of these compounds see Fig. 1.

total recoveries obtained were not sufficient to exclude the possibility that other metabolites are produced by the monkey. Chromatography of crude extracts of unhydrolyzed urine indicated that some of the pentazocine and less polar metabolites were conjugated, but no characterization of the conjugates was attempted.

Identification of metabolites. Insofar as possible, compounds recovered from incubations *in vitro* and from urine samples were characterized by thin-layer and gas–liquid chromatography and by i.r. and n.m.r. spectroscopy. Relative R_f values and relative retention times of these compounds are reported in Tables 1 and 2. We found the absolute values to vary somewhat, especially on thin-layer plates, but the relative values were generally reproducible to within 5 per cent. The thin-layer chromatography data clearly indicate the polarity of the recovered compounds to be in the following order: A < B < C < D. The greatly increased movements of compound D in acidic solvent systems suggests that it may contain a carboxylic acid group. This suggestion is consistent with the fact that the compound will not run on an OV-1 or OV-17 column unless the trimethylsilyl-derivative is prepared.

Authentic samples of 1,2,3,4,5,6-hexahydro *cis*-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6,-methano-3-benzazocine-8-ol; i.e. pentazocine (Fig. 1, a.); 1,2,3,4,5,6-hexahydro-8-hydroxy-α,6(eq), 11(ax)-trimethyl-2,6-methano-3-benzazocine-3-*trans*-2-buten-1-ol (Fig. 1, c.), and 1,2,3,4,5,6-hexahydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocine-3-crotonic acid (Fig. 1, d.) were compared to and co-chromatographed with the compounds recovered from studies *in vitro* and *in vivo* on

thin-layer and gas chromatography and found to be identical in all respects with compounds A, C and D respectively. In addition, i.r. spectra of A, C and D, with the exception of small bands due to slight impurities, were identical with the spectra of the corresponding authentic samples. The structure of these 3 compounds is, therefore, concluded to be identical to those shown in Fig. 1 a., c. and d. respectively.

Compounds B and C were similar in their susceptibility to extraction by organic solvents, their behavior in TLC systems of widely varying polarity and in their behavior upon GLC as free compounds and as acetyl- and trimethylsilyl-derivatives. In addition, the i.r. spectra of the two compounds were quite similar. It seemed likely, therefore, that B might be the geometric isomer of C. The n.m.r. spectra of B isolated from rat liver preparations and from monkey urine and of C isolated from mouse liver preparations and from monkey urine were compared to the n.m.r. spectra of authentic pentazocine (Fig. 1, a.) and its "*trans*"-alcohol (Fig. 1, c.) As expected, the spectrum of C was identical to that of the "*trans*"-alcohol and showed the expected changes when compared to the spectrum of pentazocine. The n.m.r. spectrum of B was quite similar to that of C and showed the same differences from the spectrum of pentazocine as did C. In particular, the n.m.r. spectrum of B showed the loss of 1 of the 2 methyl groups assigned to the dimethylallyl side-chain in the n.m.r. spectrum of pentazocine. It also showed the gain of a methylene group, with the chemical shift expected of a methylene group attached to an hydroxyl group, but with a shift slightly different from that of the corresponding group in C. Accordingly, metabolite B was concluded to be 1,2,3,4,5,6-hexahydro-8-hydroxy-α,6(eq),11(ax)-trimethyl-2,6-methano-3-benzazocine-3-*cis*-2-buten-1-ol (Fig. 1 b.).

The probable course of metabolism of pentazocine in the monkey, mouse and rat is shown in Fig. 1. On the basis of results obtained with other drugs, it is reasonable to assume that the hydroxylation of the methyl groups of the dimethylallyl side-chain of pentazocine is carried out by the mixed-function oxidases of hepatic microsomes and that the oxidation of one of the alcohols so produced is caused by dehydrogenases present in the soluble fraction.[9] Preliminary data obtained by one of us* indicate that compounds A, B and D are major components in the urine of human subjects given pentazocine, and that C is probably present but in much smaller amounts.

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* K. A. Pittman, unpublished results.

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