Plasma Pentazocine Radioimmunoassay

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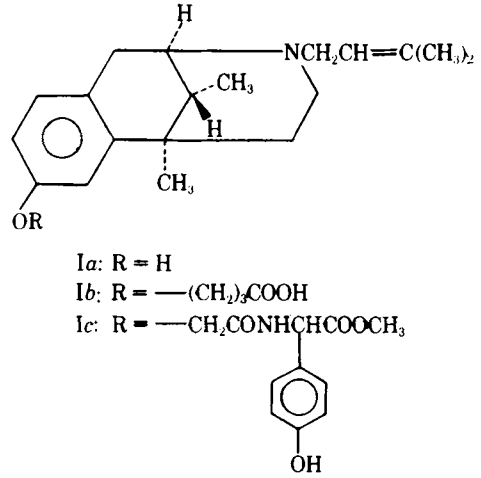
Abstract □ A sensitive and specific radioimmunoassay of dog and human plasma pentazocine is described. Rabbit antiserum and the second antibody method separated bound from free pentazocine. The radioimmunoassay employed an ^{125}I-labeled radioligand and required extraction from the sample prior to quantitation. The method had a detection limit of approximately 200 pg/assay tube (1 ng/ml). The assay was used successfully to measure pentazocine in the plasma of beagle hounds given 0.3 mg of pentazocine/kg iv. The decline in plasma levels fitted a two-compartment body model with a 100-min mean overall half-life and a 3.2-liters/hr mean plasma clearance rate.

Keyphrases □ Pentazocine—radioimmunoassay, dog and human plasma, pharmacokinetics □ Radioimmunoassay—pentazocine, dog and human plasma □ Pharmacokinetics—pentazocine, dog and human plasma, radioimmunoassay □ Analgesics—pentazocine, radioimmunoassay, dog and human plasma, pharmacokinetics

Pentazocine, (2α,6α,11R*)-(±)-1,2,3,4,5,6-hexahydro-6,11- dimethyl-3- (3-methyl-2- butenyl) -2,6- methano-3-benzazocin-8-ol[1] (Ia), is a member of the benzomorphan series of compounds (1). Pentazocine is a potent analgesic and a weak narcotic antagonist, possessing little dependence potential (2). Both oral and parenteral formulations have found widespread clinical use. To estimate the plasma pentazocine concentration accurately and conveniently, a rapid and sensitive method was needed. Previous methods include a fluorometric technique with a sensitivity of 30–50 ng/ml of plasma (3) and a GLC method with greater specificity and sensitivity (3 ng/ml) but which was more difficult to perform (4). A radioimmunoassay using ^{3}H-pentazocine (5) had no advantage in sensitivity over the GLC method.

This study reports a new radioimmunoassay, using a ^{125}I-labeled pentazocine derivative, with sensitivity in the subnanogram range. This assay has been used to measure pentazocine in both human and dog plasma. The technique permitted determination of pentazocine pharmacokinetic parameters in dogs that received a 0.3-mg/kg iv dose.



Ia: R = H
Ib: R = —$(CH_2)_3$COOH
Ic: R = —$CH_2CONHCHCOOCH_3$

EXPERIMENTAL

Solutions—Iodination buffer contained 0.5 *M* Na_2HPO_4–0.5 *M* NaH_2PO_4 (4:1), adjusted to a final pH of 7.5. Dilute iodination buffer was the same buffer diluted 1:10 with water. Phosphate-buffered saline was prepared by dissolving 24.51 g of NaCl, 1.77 g of $NaH_2PO_4 \cdot H_2O$, 3.21 g of Na_2HPO_4, and 0.285 g of thimerosal[2] in 3 liters of distilled water and adjusting the pH to 7.0. The assay buffer contained 0.1% (w/v) gelatin dissolved in phosphate-buffered saline. Nonimmune normal rabbit serum was diluted 1:400 in phosphate-buffered saline containing 0.05 *M* edetate sodium[3].

Antipentazocine Serum Preparation—A carbodiimide condensation (6) conjugated a pentazocine hapten[4] (Ib) with bovine serum albumin basic amino acid residues. Approximately 17 hapten molecules were conjugated to each albumin molecule, as shown by a trinitrobenzenesulfonic acid free amino group determination (7). The hapten-protein conjugate (2.0–3.0 mg/rabbit) was emulsified in complete Freund's adjuvant[5] and injected intradermally at several sites on the backs of seven Dutch belted rabbits (8). Conjugate (1.0 mg/rabbit) in incomplete Freund's adjuvant was administered as a booster immunization at approximately 3-week intervals for 3 months. Serum was obtained from blood collected from rabbit marginal ear veins.

Second Antibody Preparation—Rabbit γ-globulin[6] (100 μg), emulsified in complete Freund's adjuvant, was injected subcutaneously at monthly intervals into an adult female goat to obtain anti-rabbit γ-globulin. The goat was bled 2 weeks after each immunization, and serum was obtained. The appropriate second antibody dilution was determined by a progesterone assay[7]; a 1:16 dilution was used.

Radioiodination—At room temperature, a methyl tyrosinate pentazocine derivative[8] (Ic) was radiolabeled by a modified method (9). Sodium ^{125}I-iodide[9] (1.0 mCi) was added to a vial containing 50 μl of iodination buffer and 10 μl of the methyl tyrosinate derivative (1 mg/ml of methanol). The vial was stoppered, and the contents were gently agitated. Thirty micrograms of chloramine-T[10] in 15 μl of dilute iodination buffer was added, and the mixture was allowed to react for 2 min. Then 60 μg of sodium metabisulfite in 30 μl of dilute iodination buffer was added to the vial to stop the reaction.

A solution (100 μl) containing 16% (w/v) of sucrose dissolved in assay buffer was added, and the sample was transferred to a 0.5 × 20-cm gel filtration[11] column, which had been equilibrated with assay buffer. Then 70 μl of 8% (w/v) sucrose dissolved in assay buffer was used to rinse the vial, and the wash solution was added to the column. The column was eluted with assay buffer, and 1.0-ml fractions were collected and counted to determine the radioactivity.

Extraction Procedure—Duplicate samples (0.1 or 0.2 ml) of pentazocine-containing plasma were added to extraction tubes (15 × 85 mm). Pentazocine standards (1–100 ng/ml) were included. Water (0.8 or 0.9 ml) and saturated sodium bicarbonate–sodium carbonate extraction buffer (0.1 ml) were added to the plasma in each extraction tube. Ben-

[1] Talwin, Winthrop Laboratories, New York, N.Y.

[2] Sigma Chemical Co., St. Louis, Mo.

[3] Ethylenediaminetetraacetic acid, sodium salt, Sigma Chemical Co., St. Louis, Mo.

[4] 4-[1,2,3,4,5,6- Hexahydro-*cis*-6,11-dimethyl-3- (3-methyl-2- butenyl)-2,6-methano-3-benzazocin-8-yloxy]butanoic acid.

[5] Difco Laboratories, Detroit, Mich.

[6] Fraction II, Miles Laboratories, Kankakee, Ill.

[7] J. E. Peterson and G. D. Niswender, unpublished data.

[8] Methyl *N*-(2-[1,2,3,4,5,6-hexahydro-*cis*-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6- methano-3-benzazocin-8- yl]oxy]-1-oxoethyl)tyrosinate hydrochloride.

[9] New England Nuclear, Boston, Mass.

[10] Eastman Kodak, Rochester, N.Y.

[11] Sephadex G-25-150, Sigma Chemical Co., St. Louis, Mo.

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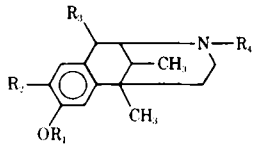
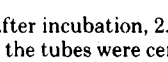
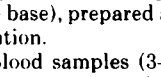
Table I—Relative Affinities of Pentazocine and Related Compounds to the Antiserum



Number	R_1	R_2	R_3	R_4	Relative Affinity
I	H	H	H	$-CH_2CH=C(CH_3)_2$	1.000
II	H	H	H	$-CH_2C(-CH_3)=CH(CH_3)_2$	0.045
III	$-CH_2COOH$	H	H	$-CH_2CH=C(CH_3)_2$	0.032
IV	H	H	H		0.008
V	H	H	H	$-CH_2CH=CH_2$	0.007
VI	H	H	H	$-CH_2CH_2CH_3$	0.007
VII	H	H	H	$-CH_2-CH=C(-CH_3)-CH_2OH$	0.006
VIII	H	H	=O		0.003
IX	H	H	H	H	0.002
X	H	H	H	$-CH_2CH=C(-CH_3)-COOH$	0.001

zene[12] (8 ml) was added, and the tubes were shaken mechanically for 15 min. Then tubes were centrifuged for 5 min at 2200×*g*, and duplicate benzene aliquots were removed and placed in appropriately labeled tubes (12 × 75 mm) for the assay. The benzene was evaporated under a nitrogen stream, and the residue was dissolved in 0.5 ml of assay buffer. The tubes were mixed, stoppered, and left overnight at 4°.

Radioimmunoassay—The pentazocine radioimmunoassay was similar to procedures reported for steroids (10). The antipentazocine serum (200 μl), at a dilution of 1:15,000 in normal rabbit serum, was added to the assay tubes containing the plasma extracts. Radioiodinated tyrosinate pentazocine derivative (25,000–30,000 cpm/100 μl) was added. The tubes were incubated for 4–6 hr at 4°. Appropriately diluted second antibody (200 μl) was added, and the incubation was continued overnight.

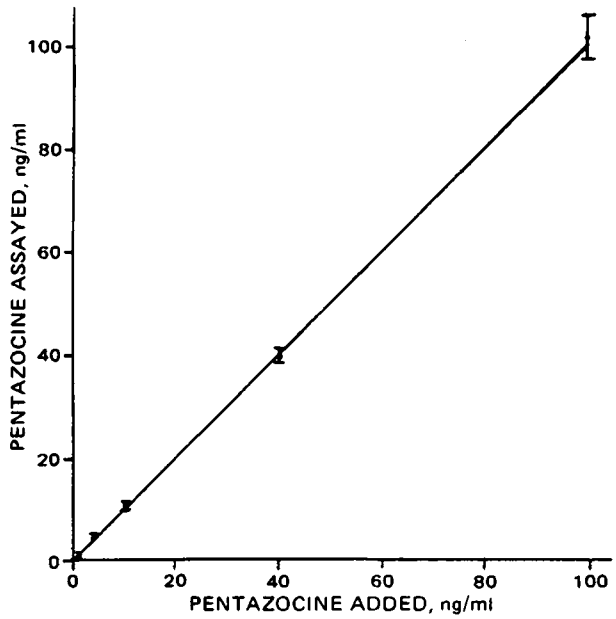


Figure 1—*Correlation of pentazocine added to human plasma with the mean values obtained by radioimmunoassay. Slope = 1.020, intercept = 0.034, and r (correlation coefficient) = 0.9705. The short vertical lines represent 2* SE (n = *8).*

[12] Analytical reagent, Mallinckrodt, St. Louis, Mo.

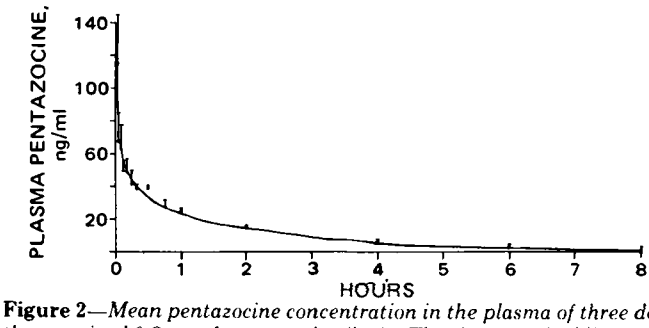


Figure 2—*Mean pentazocine concentration in the plasma of three dogs that received 0.3 mg of pentazocine/kg iv. The short vertical lines represent 1* SE.

After incubation, 2.5 ml of cold phosphate-buffered saline was added, and the tubes were centrifuged at 2500 rpm for 30 min. The supernatant solutions were discarded, and the precipitates were counted. Duplicate standards (1–100 ng/ml) were included in each assay run.

Dog Study—One male (12.6 kg) and two female (9.6 and 17.2 kg) beagle hounds were given 0.3 mg of pentazocine/kg iv (calculated as the free base), prepared as a 3-mg of pentazocine/ml of physiological saline solution.

Blood samples (3–5 ml) were collected from an indwelling venous catheter from 1.0 min to 8 hr after administration. Potassium oxalate was the anticoagulant. The samples were centrifuged, and the plasma was frozen until analyzed.

RESULTS AND DISCUSSION

All seven rabbits produced antiserum within 6 months; the antiserum titers ranged from 1:1000 to 1:20,000. The 1:15,000 antiserum dilution (~1:75,000 final dilution in the assay tube) resulted in 50–55% radioligand binding.

The antiserum specificity was assessed by three related methods. First, the ability of several related compounds, including known pentazocine metabolites, to compete with the radioiodinated pentazocine tyrosinate derivative for antibody binding was determined by comparing the amount of each compound required for 50% radioactivity binding inhibition with the pentazocine standard curves (11). Second, the percent displacement of the related compounds was evaluated (12). Third, the percent cross-reactivity of the related compounds was determined in extracted plasma.

The antiserum exhibiting the least cross-reactivity was selected for all subsequent work. The relative affinities of the related compounds for this antiserum are shown in Table I. The two compounds (II and III) that demonstrated a slight cross-reactivity with pentazocine (I) are not metabolites and posed no problem. The known metabolites (13) of pentazocine (*trans*-acid, *trans*-alcohol, and norpentazocine; X, VII, and IX, respectively) showed little affinity for this pentazocine antiserum.

The assay sensitivity or detection limit (14) is the smallest compound amount that is significantly different from zero at the 95% confidence limit. The human plasma blank gave a detection limit of 0.21 ng/assay tube. The dog plasma blanks gave a limit of 0.19 ng/tube. These values correspond to about 1 ng of compound/ml of plasma.

Precision was tested by calculating the within-assay and between-assay variances. The within-assay variance was evaluated by duplicate measurements of the same sample, and the between-assay variance was evaluated by duplicate measurements in different assays using samples with a wide range of concentrations. The variation coefficient from the mean duplicate determinations was estimated (15). The within-assay variation coefficient ranged from 7.4 to 10.1% (mean 8.2%, $n = 18$). The between-assay variation coefficient ranged from 10.5 to 13.2% (mean 11.6%, $n = 3$). These values are within the range reported for steroid radioimmunoassays (11).

Exogenous pentazocine was accurately quantitated in human plasma by the pentazocine radioimmunoassay system (Fig. 1). A similar correlation was found for canine plasma. No statistical difference was found between canine and human plasma standards.

The mean plasma pentazocine concentrations in the three dogs are shown in Fig. 2. Plasma levels declined biexponentially with time, suggesting that a two-compartment model could be applied. Pharmacokinetic parameters were computer fitted by a modified program (16) (Table II). Previous work indicated that the two-compartment model was applicable to pentazocine pharmacokinetics in dogs (17). The re-

Table II—Pharmacokinetic Parameters for the Disappearance of Dog Plasma Pentazocine following an Intravenous Dose of 0.3 mg/kg

Animal Weight, kg, and Sex	Rate Constants, hr^{-1}			Plasma Clearance Rate, liters/hr	Apparent Distribution, Volume, Steady State, liters	$t_{1/2}^{a}$, min	Area under the Curve, ng hr/ml
	K_{12}	K_{21}	K_{10}				
17.2 F	2.27	1.89	1.05	2.88	6.04	100.2	99.6
9.6 F	2.02	3.41	0.65	3.99	9.80	106.8	80.7
12.6 M	14.6	5.26	1.67	2.68	6.07	99.6	107
Mean	6.29	3.52	1.12	3.18	7.30	102.2	95.9
±*SEM*	±4.15	±0.97	±0.29	±0.41	±1.25	±2.3	±7.95

[a] Calculated from $\frac{0.693}{1/2[(K_{12}+K_{21}+K_{10})-\{(K_{12}+K_{21}+K_{10})^2-4K_{12}K_{21}\}^{1/2}]}$.

ported pentazocine terminal elimination half-life of 150 min in dogs is in good agreement with the present estimate (102 min, Table II). The two-compartment model was used recently to describe pentazocine pharmacokinetics in healthy humans (18) where the average terminal disposition half-life was 203 min.

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Drug Entrapment for Controlled Release in Radiation-Polymerized Beads

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Abstract □ The preparation of beads including polymer, vinyl monomer, and drug was carried out by low temperature, radiation-induced polymerization. Complete spherical particles were obtained when ethanol was the precipitation medium. Polymers such as polymethyl methacrylate and polystyrene were dissolved in various glass-forming monomers, and the drug was dispersed in the mixture. The mixture was dropped into cold precipitation media. The formed monomeric particle was irradiated at low temperatures to produce polymerization. The drug release profiles from polymerized particles were changed by varying the glass-forming monomer and the precipitation medium.

Keyphrases □ Dosage forms—controlled-release delivery devices, radiation-polymerized beads, potassium chloride □ Potassium chloride—controlled-release delivery devices, radiation-polymerized beads □ Polymers—controlled-release delivery devices, beads, potassium chloride

Controlled drug release may improve effectiveness and safety in drug use (1–3). Drug entrapment in polymerized matrixes may be an effective way to control release. Various bioactive materials, such as enzymes or microbial cells, could first be attached effectively to a carrier surface and then incorporated into a polymer by means of radiation-induced polymerization at low temperatures (4, 5). This method can be performed using monomers that form stable glass-like states when supercooled and that can be readily polymerized at low temperatures (6).

This method can be applied to entrapping low molecular weight drugs for controlled release as well as high molecular weight bioactive substances. Entrapping drugs in fine polymer particles, such as microspheres, could have practical advantages. Trapping enzymes or microbial cells on a carrier surface is desirable for the contact with the substrate (7). Immobilized enzyme or microbial cell microspheres were obtained by suspension polymerization of a hydrophobic glass-forming monomer and a buffered enzyme solution at low temperatures or by polymerization

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