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Pharmacokinetics, Metabolism, and Urinary Excretion of [^{3}H]Alphaprodine in Dogs†

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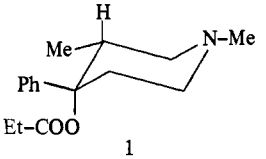
The physiologic disposition of the narcotic analgetic, alphaprodine (1), following iv adminstration in dogs conforms to a two-compartment, open-system model. The rapid disappearance of 1 from plasma and the large volume of distribution are in keeping with its rapid onset and short duration of action. The only extractable metabolite, noralphaprodine (2), achieved maximum plasma levels within 40 min after injection and remained constant for the duration (4 hr) of the experiment. The urine was found to contain 2 and conjugated metabolites whose identity presently is not known. Evidence is presented which suggests there is rapid equilibration between brain and plasma.

Alphaprodine‡ (1) is a potent narcotic analgetic which possesses a rapid onset and a short duration of action.[1,2] It is useful in obstetrics and surgical procedures where rapid analgesia of short duration is desired.[2] Metabolism and distribution studies in mice after sc administration suggested that the quick onset and offset of action of 1 is related to its rapid penetration into the brain and its rapid disappearance from plasma.[3,4] Investigation in man indicated that after iv injection of 1, the plasma levels do not decline in a monoexponential fashion, but not enough data points were collected to allow conclusions regarding the nature of the pharmacokinetic model.[5] As the duration of action[6] of 1 appears to be correlated with its rapid, initial decline in the plasma,[5] it was of interest to determine the pharmacokinetic parameters in an animal model. This report describes the pharmacokinetic profile, metabolism, and urinary excretion of 1 in dogs.



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Experimental Section

Materials. The syntheses of [^{3}H]alphaprodine·HCl (1·HCl), 1-propionyl-3-methyl-4-phenylpiperidin-4-ol (3), and 3-methyl-4-phenylpiperidin-4-ol (5) have been described previously.[4] (±)-1,3-Dimethyl-4-phenylpiperidin-4-ol (4) was obtained from Hoffmann-LaRoche, Inc. β-Glucuronidase, type H-2 from Helix Pomatia (Sigma Chemical Co.) containing approximately 13400 IU of β-glucuronidase and 6300 μ*M* units of arylsulfatase activity per ml, was diluted 200-fold with distilled H_2O and used for the enzymatic hydrolysis of conjugates.

Protocol. Two male mongrel dogs weighing 23.6 and 25.9 kg each received single iv doses of 2 and 1 mg/kg of [^{3}H]alphaprodine·HCl in normal saline. Heparinized blood samples were collected over the next 4 hr and the plasma was separated from red cells by centrifugation and analyzed immediately. During the first 4 hr after injection urine samples were collected through a catheter that had been inserted in the urinary bladder. The catheter was then removed, the animals placed in separate metabolic cages, and the urine collected for 24 hr. The pH values of urine samples were determined immediately after collection and the samples were frozen until analyzed.

Extraction Procedure. Plasma or urine (1 ml) was adjusted to pH > 12 with KOH, allowed to stand for 5 min, and mixed with twice its volume of C_6H_6. After shaking and centrifugation, an aliquot of the C_6H_6 phase was transferred to a centrifuge tube, mixed with 0.1 *N* HCl, shaken, and centrifuged. The C_6H_6 phase, after washing with 0.1 *N* HCl, was used for analysis of the N-demethylated metabolite. The aqueous acid phase was washed with C_6H_6, basified, and extracted with C_6H_6. The C_6H_6 phase was used for the analysis of the unchanged drug.

Acid Hydrolysis of Conjugates in Urine. Urine was extracted with benzene, neutralized with dil HCl, and mixed with 0.25 its volume of concd HCl. The sample was autoclaved (121° at 18 psi) for 80 min, adjusted to pH > 12 with KOH, and mixed with twice its volume of C_6H_6. After shaking and centrifugating, an aliquot of the C_6H_6 phase was used for analysis of conjugated metabolites.

Enzymatic Hydrolysis of Conjugates in Urine. After extraction with benzene, urine was adjusted to pH 7 with dil HCl and mixed with an equal volume of 0.1 *M* acetate buffer (pH 5.4). The mixture was shaken at 37° for 20 hr with β-glucuronidase (300 IU/ml of urine) and adjusted to pH > 12 with KOH. After shaking with twice its volume of C_6H_6, the mixture was centrifuged and an aliquot of the C_6H_6 phase was used for analysis of conjugated metabolites.

Analysis of Extracts. Radioactivity was measured in a Packard Tri-Carb Model 3375 liquid scintillation spectrometer. The C_6H_6 solutions to be analyzed were transferred to glass counting vials and mixed with 10 ml of scintillation cocktail consisting of 5.5 g of Permablend I (Packard Instrument Co.) in 1 l. of toluene. Controls with different known concentrations of the labeled drug were run concurrently with the experiment to serve as a check on the overall technique. All samples were counted for sufficient time to yield <2.5% error. The identity of the radioactive material in the benzene extracts was determined by tlc on basic alumina and ethyl acetate or $CHCl_3$–C_6H_6 (75:25 saturated with NH_4OH) as solvents.

Total Radioactivity in Urine. Urine (1 ml) was transferred into a glass counting vial, mixed with 1.5 ml of NCS (Amersham/Searle), and diluted with 10 ml of scintillation cocktail. The radioactivity in the samples was measured in a liquid scintillation spectrometer.

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‡Marketed as Nisentil by Hoffmann-LaRoche.

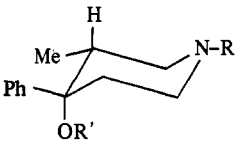
Control samples of urine containing different known concentrations of the labeled drug were run concurrently with the experiment to determine the counting efficiencies.

Identification of Urinary Metabolites. An aliquot (200 ml) of urine was adjusted to pH > 12 with KOH and extracted with twice its volume of $CHCl_3$. The extraction was repeated several times and the combined $CHCl_3$ extracts were concentrated under reduced pressure. The residue was dissolved in 1 ml of acetone and analyzed by glc and tlc. The analysis was performed at 160° on a Varian 2100 gas chromatograph equipped with a flame ionization detector and a 1.8 m × 6.35 mm o.d. U-shape glass column packed with 3% OV-17 on chromosorb W (80–100 mesh). The retention times were: 1, 7.5 min; 3, 45 min; 4, 3.2 min; and 5, 4.3 min. Thin-layer chromatography was run on basic alumina sheets (Eastman chromagram 6062) and silica gel sheets (Eastman chromagram 6060) in one dimension. The solvent systems employed were $CHCl_3$–C_6H_6–EtOH–1.0 *M* NH_4OH (60:40:1:1), ethyl acetate and $CHCl_3$–C_6H_6 (75:25 saturated with NH_4OH) with alumina sheets, and $CHCl_3$–MeOH (85:15) with silica gel sheets.

Results and Discussion

The plasma level curves following iv doses of alphaprodine (1) are presented in Figures 1 and 2. The disappearance of 1 from plasma followed a biexponential pattern and the pharmacokinetic parameters summarized in Table I were calculated for a two-compartment, open-system model, with elimination occurring from the central compartment.[7] It is apparent that 1 distributed very rapidly into tissue outside the vasculature, as the volume constant (V_p) of the central compartment is several times larger than the total plasma volume (average plasma volume is 55.2 ml/kg body weight).[8] This phenomenon, which has been reported for other drugs,[9] can be attributed to the high lipid solubility of alphaprodine (octanol-pH 7.4 buffer partition coefficient is 10.55[4]) and might explain the rapid onset of its analgetic effect after iv administration.

Determination of plasma and urine levels of N-demethylated metabolite 2 was carried out by base-promoted, intramolecular O → N propionyl migration of 2 to amide 3, which was easily separated from 1 by a simple extraction procedure.[4] Small amounts of the N-demethylated metabolite were detected in plasma as early as 4 min after injection. Figure 2 represents the plasma levels of 1 and its N-demethylated metabolite at different time intervals after iv injection. It is apparent that the plasma level of 2 increased during the first 40 min after administration and thereafter maintained a constant level for the time period measured. A similar pattern of metabolite accumulation in plasma has been observed with other drugs[10] and has been attributed to the slower rate of metabolite excretion compared to that of the parent drug.[11]



2, R = H; R' = COEt
3, R = COEt; R' = H
4, R = Me; R' = H
5, R = R' = H

Investigation of the $CHCl_3$-extractable urinary excretion products indicated the presence of alphaprodine (1) and the rearranged compound (3) derived from N-demethylated metabolite 2. It is noteworthy that the same metabolite also was detected in mice. There was no evidence for the

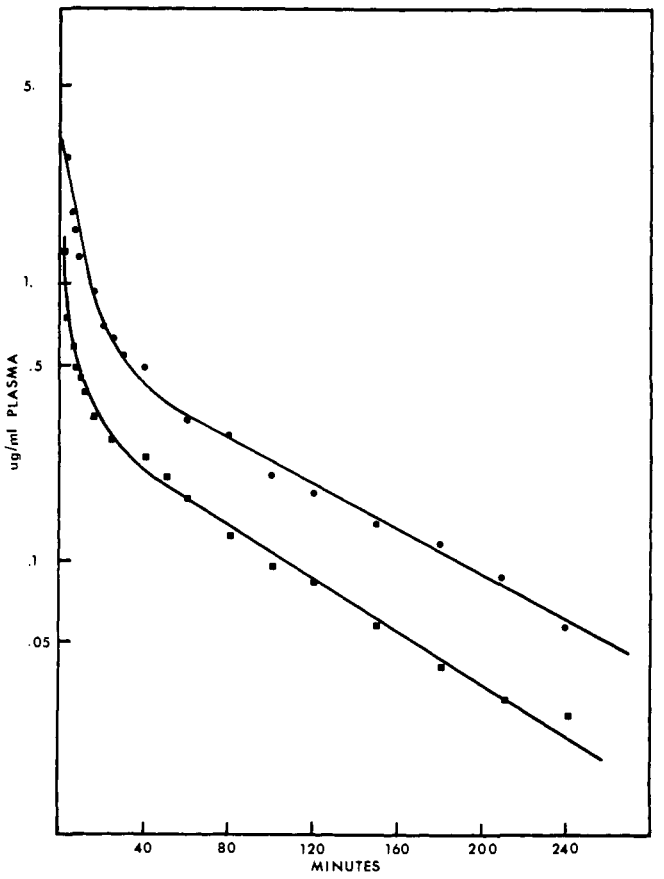


Figure 1. Semilogarithmic plots of plasma levels of [^{3}H]alphaprodine in dog 1 at different times after intravenous doses of 2 mg/kg (●) and 1 mg/kg (■).

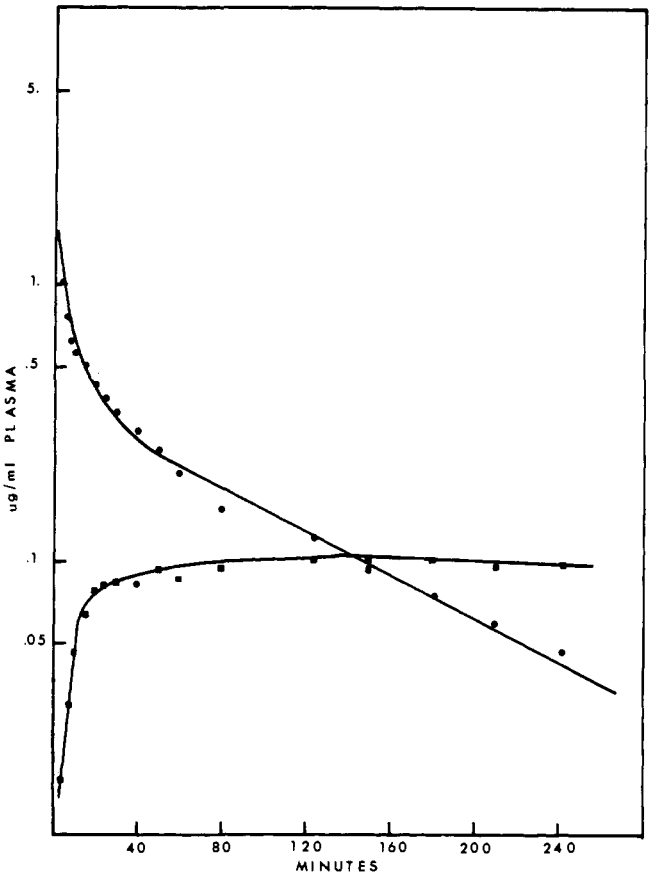


Figure 2. Semilogarithmic plots of levels of [^{3}H]alphaprodine (●) and N-demethylated metabolite 2 (■) in plasma of dog 2 at different times after a 2 mg/kg intravenous injection.

Table I. Pharmacokinetic Parameters[a] Calculated from Data Obtained after Intravenous Injection of Alphaprodine·HCl in Dogs

	Dog 1		Dog 2	
	2 mg/kg	1 mg/kg	2 mg/kg	1 mg/kg
A,[b] μg/ml	2.8	1.4	1.15	1.0
B,[b] μg/ml	0.59	0.33	0.87	0.375
α,[b] min^{-1}	0.116	0.173	0.099	0.107
Half-life for α, min	6.0	4.0	7.0	6.5
β,[b] min^{-1}	0.0094	0.011	0.0072	0.0089
Half-life for β, min	74.0	63.0	96.0	78.0
C_p^0,[c] μg/ml	3.39	1.73	2.02	1.375
Rate constants, min^{-1}				
k_{el}[d]	0.038	0.045	0.015	0.027
k_{12}[e]	0.059	0.097	0.044	0.053
k_{21}[e]	0.028	0.042	0.047	0.036
Volumes of distribution, liters[f]				
V_{dss}	36.7	38.4	38.3	35.8
V_p	11.8	11.6	19.8	14.5
V_t	24.9	26.8	18.5	21.3

[a] Calculated for a two-compartmental, open-system model.[7] [b] The general equation used is $C_p = Ae^{-\alpha t} + Be^{-\beta t}$, where C_p is the concn of drug in plasma, A and B are coefficients, and α and β are hybrid rate constants. [c] C_p^0 is the concn of drug in plasma at zero time, $C_p^0 = A + B$. [d] The elimination rate constant (k_{el}) is the sum of the simultaneous processes of metabolism and excretion all of which are assumed to be first order. [e] k_{12} is the first-order rate constant of distribution of the drug from the central to the peripheral compartment; k_{21} is the rate constant for the reverse process. [f] V_{dss}, V_p, and V_t are the steady-state volume of distribution, volume of the central, and volume of tissue compartments, respectively.

Table II. Urinary Excretion of Alphaprodine and Metabolites after Intravenous Injection in Dog 1

	Cumulative per cent of the injected dose excreted					
	Total radioactivity[b]		Unchanged drug (1)[c]		Nor compound (2)[d]	
Time,[a] hr	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
1	6.8	5.9	0.9	0.4	0.8	0.6
2	14.1	14.7	1.4	1.5	1.4	1.3
3	21.0	22.0	2.0	2.9	1.5	2.0
4	27.5	28.9	2.4	4.0	1.9	2.8
24	36.0	48.4	2.9	6.9	5.0	6.2

[a] Time after iv administration. [b] Determined by direct counting of radioactivity in urine. [c] Extracted from urine as the basic fraction and identified by tlc. [d] Extracted from urine as the nonbasic fraction and identified by tlc.

presence of potential metabolites **4** and **5**.

The per cent of the administered dose excreted as **1** and **2** in the urine of dog 1 (Table II) indicates that only a small fraction of the drug was recovered unchanged and that the major extractable metabolite was the N-demethylated compound.

As the total amount of **1** and **2** accounted for only 22–25% of the total radioactivity in urine, the nature of the nonextractable metabolite was investigated. The remainder of the radioactivity could be extracted after acid hydrolysis, thus suggesting the presence of conjugated metabolite(s). Glc and tlc showed two major and two minor components, none of which was identical with known compounds. An enzyme preparation which contained β-glucuronidase and arylsulfatase activities hydrolyzed 40% of the acid-hydrolyzable conjugates. The constitution of these conjugates is currently under investigation.

The detailed pharmacokinetic study in dogs suggests that the disappearance of alphaprodine in man can be described by a two-compartmental, open-system model where the initial, rapid exponential decline in plasma levels is due to a distribution of the drug into tissues of the peripheral compartment. Since the rapid onset (1–2 min) and short duration (30–45 min)[6] of analgesia in man corresponds to the time period where the initial decline[5] of alphaprodine in plasma occurs, it appears that rapid equilibration between brain and plasma takes place and that the termination of analgesia is due to tissue distribution. Thus, from a practical point of view, brain and plasma may be considered a single compartment for this drug.

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