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Pharmacokinetics of Naloxone and Naltrexone in the Dog[1, 2]

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ABSTRACT

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The serum kinetics of 5 mg/kg of i. v. naloxone or naltrexone were studied in each of two groups of five dogs; serum samples were obtained from 2 min to 2 hr after injection. Serum concentrations were determined by radioimmunoassay. Serum levels of both naloxone and naltrexone fell rapidly; serum half-life during the elimination phase was 71.2 ± 8.9 min (mean ± S.E.) for naloxone and 85.1 ± 9.0 min (mean ± S.E.) for naltrexone. Although human and dog kinetics are similar for naloxone, naltrexone is long-acting in man, but is quickly dissipated in the dog.

Naloxone is a short-acting pure narcotic antagonist whose pharmacokinetics are known for rat and man (Ngai *et al.*, 1976). Naltrexone is a N-cyclopropyl derivative of naloxone with greater potency and longer duration in man and has been evaluated as a chemotherapeutic agent for the treatment of opiate-dependence (Verebey and Mulé, 1975).

The dog is a commonly used experimental animal in the study of hemodynamic and central nervous system consequences of narcotic antagonists (Martin and Sandquist, 1974; Patschke *et al.*, 1977). Thus, we studied the pharmacokinetics of naloxone and naltrexone in the dog.

Methods

Two groups of five fasted, unmedicated, mongrel dogs of either sex, weighing about 10 kg, were anesthetized with halothane; the tracheas were intubated and the dogs were mechanically ventilated with 100% O_2 to maintain a mild respiratory alkalosis; pH 7.46 ± 0.04 (mean ± S.D.), pCO_2 27 ± 6 torr (mean ± S.D.). An arterial cannula was placed for blood sampling, and a peripheral intravenous catheter was inserted for administering drugs and fluids. End-tidal gas was sampled from the endotracheal tube and continuously monitored for carbon dioxide and halothane by two infrared gas analyzers. After initial instrumentation and stabilization, end-tidal halothane was kept between 1.1 to 1.25%. A rectal thermometer was inserted and temperature was maintained between 37.0–38.5°C with heating lamp and blanket.

Naloxone, 5 mg/kg, and naltrexone, 5 mg/kg, were each given to five dogs as a rapid i. v. bolus. Arterial blood was collected at 2, 5, 10, 15, 30, 60 and 120 min. The blood was allowed to clot, and the serum was frozen until analyzed.

The radioimmunoassay for naloxone and naltrexone, modified from Berkowitz *et al.* (1975) was performed as follows:

1. Tubes for standards were prepared containing either 0.2 to 2.0 ng of naloxone or 0.4 to 4.0 ng of naltrexone in 0.1 ml of normal human serum (NHS). Assay tubes were prepared with 0.1 ml of serum sample diluted in NHS.
2. Rabbit antiserum to naloxone was diluted 1:140 in 0.05 M Na_2HPO_4, 0.85% NaCl, 1% bovine serum albumin, pH 7.0 [phosphate buffered saline-bovine serum albumin (PBS-BSA)] and 0.1 ml of the diluted antiserum was added to each tube (a control tube contained PBS-BSA instead of antiserum).
3. [^{3}H]Naloxone (10 μCi/0.5 nmol/ml) was diluted 1:60 in PBS-BSA and 0.3 ml of the diluted isotope was added to each tube. The tubes were incubated at room temperature for 60 min.
4. The antibody-bound antigen was then precipitated by the addition of 0.5 ml of saturated $(NH_4)_2SO_4$, pH 7.3.
5. The precipitates were centrifuged and washed once with a mixture of equal volumes of saturated $(NH_4)_2SO_4$, pH 7.3, and PBS-BSA.
6. The washed precipitates were then dissolved in 1.0 ml of distilled water, and the radioactivity was determined in a liquid scintillation spectrometer using a cocktail consisting of 3 parts toluene containing 5.33 g of Omnifluor (New England Nuclear Corp., Boston, Mass.) per liter of toluene plus 1 part Triton X-100 (New England Nuclear); 1.0 ml of aqueous sample was added to 14 ml of cocktail for scintillation counting.
7. Standard curves were obtained for naloxone and naltrexone. An example is shown in figure 1.

The serum decay curves for naloxone and naltrexone were fitted to polyexponential equations of form

$$C = \sum_{i=1}^{n} C_i e^{-\lambda_i t}$$

by a back projection technique (Wagner, 1975). Serum half-lives and serum concentrations were compared by a one-way analysis of variance.

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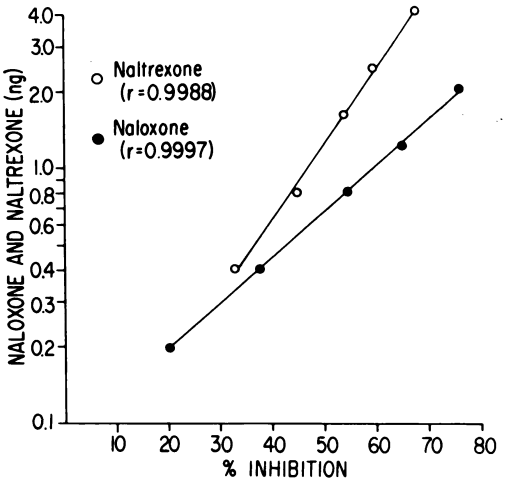

Fig. 1. Inhibition of binding of naloxone[^{3}H] to rabbit antiserum by nonradioactive naloxone and naltrexone in serum. The lines are a least squares exponential curve fit.

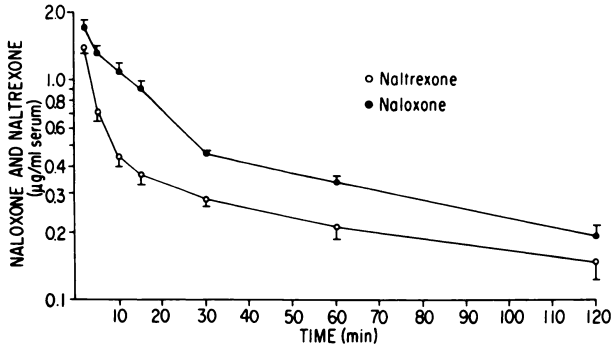

Fig. 2. Comparative serum disposition of naloxone and naltrexone in dogs after a dose of 5 mg/kg i.v. Results are the average ± S.E. of five dogs.

Results

The serum concentration (micrograms per milliliter) of naloxone following a 5 mg/kg i.v. bolus was best described by the biexponential equation $C = 1.2162\ e^{-0.0815t} + 0.5478\ e^{-0.0096t}$ (t = min). Naltrexone disappearance was characterized by $C = 1.7989\ e^{-0.3085t} + 0.3808\ e^{-0.0085t}$ (fig. 2). The serum half-life for the elimination phase was 71.2 ± 8.9 min (mean ± S.E.) for naloxone and 85.1 ± 9.0 min for naltrexone. These half-lives were not significantly different ($F_{(1,8)} = 1.20$, $P > .2$). During the initial distribution phase the serum half-lives for naloxone and naltrexone were 8.9 ± 0.9 and 2.4 ± 0.3 min, respectively; these were significantly different ($F_{(1,8)} = 43.1$, $P < .002$). The serum concentration of naloxone was significantly higher than naltrexone serum concentrations from 2 to 60 min (table 1).

Discussion

This radioimmunoassay is quite sensitive for naloxone or naltrexone, but the antibody also recognizes a metabolite of naloxone (reduction of the C-6 ketone group to produce hydroxynaloxone, "naloxol") (Berkowitz et al., 1975). Although not studied, it is probable that the equivalent metabolite of naltrexone (naltrexol) is also recognized (B. A. Berkowitz, personal communication). In contrast, the N-dealkylated and conjugated metabolites of naloxone are not recognized (Berkowitz *et al.*, 1975); the similar metabolites of naltrexone are likewise probably not recognized (B. A. Berkowitz, personal communication). In the dog naltrexone is metabolized by conjugation with very small amounts of naltrexol also produced (Cone *et al.*, 1974a; Cone and Gorodetzky, 1976). We cannot exclude the presence of naloxol in the dog as naloxone metabolism has not been studied in this species; however, it is not likely to be an important metabolite (Berkowitz *et al.*, 1975). Thus, there should be no metabolites interferring with the radioimmunoassay, but confirmation by more specific methods is needed.

TABLE 1
Serum Concentrations of naloxone and naltrexone

Time	Naloxone (Mean ± S.E.)	Naltrexone (Mean ± S.E.)	$F_{(1,8)}$	P
min	*μg/ml*	*μg/ml*		
2	1.732 ± 0.096	1.390 ± 0.078	7.68	< .05
5	1.320 ± 0.086	0.724 ± 0.079	26.14	< .002
10	1.102 ± 0.092	0.446 ± 0.047	40.39	< .002
15	0.915 ± 0.071	0.368 ± 0.041	44.21	< .002
30	0.462 ± 0.012	0.283 ± 0.019	65.03	< .002
60	0.342 ± 0.026	0.215 ± 0.026	11.95	< .02
120	0.197 ± 0.020	0.149 ± 0.024	2.44	> .2

The kinetics of naloxone in dogs has similarities with previous work. In the rat, 5 mg/kg of i.v. naloxone produced a serum concentration of 1.45 ± 0.1 μg/ml at 5 min (Ngai *et al.*, 1976); in the present study, the concentration was 1.320 ± 0.086 μg/ml at 5 min. However, the half-lives during elimination were 30 min in the rat and 71.2 min for the dog. The half-lives in man and in the dog were quite similar, 64 ± 12 min vs. 71.2 ± 8.9 min, respectively (Ngai *et al.*, 1976).

In the rat, naloxone and naltrexone have essentially identical serum decay curves after i.v. injection (Berkowitz *et al.*, 1976). Thus, naltrexone serum kinetics are quite similar in rats and dogs. Our naltrexone kinetics are not comparable to studies in man as the latter have been done after acute and chronic oral naltrexone administration and over 24 to 72 hr (Cone *et al.*, 1974b; Verebey *et al.*, 1976; Kogan *et al.*, 1977). After oral administration, there is considerable first-pass hepatic metabolism (75% dose) (Kogan *et al.*, 1977).

In many species naltrexone is more potent and longer acting than naloxone. In the rat naltrexone has twice the potency and 3 times the duration (Verebey and Mulé, 1975). In man a similar greater potency and longer duration have been observed (Verebey and Mulé, 1975). However, in the dog while the potency of naltrexone is twice that of naloxone, the durations are the same (Martin *et al.*, 1973; Martin and Sandquist, 1974). The longer duration of naltrexone over naloxone might be due to a prolonged half-life, a longer retention of naltrexone in the brain or the presence of an active metabolite.

Berkowitz *et al.* (1976) compared serum and brain concentrations of naloxone and naltrexone in the rat after a 5 mg/kg i.v. dose; there were no major differences between the serum or brain profile of these drugs. Since the plasma protein binding of naltrexone is the same for man, rat and dog (about 20–25% bound) and is also constant over a wide range of concentrations (Ludden *et al.*, 1976), it is unlikely that the different durations of naltrexone action between dog and man and rat is due to longer cerebral retention.

As noted above, our results showed similar naltrexone kinetics in the dog as shown previously in the rat. Although not

strictly comparable, the urine and serum profile in man after oral naltrexone revealed a half-life of 1.1 hr (Cone *et al.*, 1974b) and a primary half-life of 3 hr (Verebey *et al.*, 1976), respectively. This is not too dissimilar from the half-life of 1.4 hr in dog. Thus, the prolonged effect of naltrexone in man and rat and not in dog is probably not explained by a difference in half-life.

An active metabolite (naltrexol) has been found in some, but not in all species. Naltrexol has 1/50 to 1/12 the potency of naltrexone (Verebey and Mulé, 1975; Cone *et al.*, 1974b). In man the major biotransformation product of naltrexone is naltrexol with eventual urinary excretion (Cone *et al.*, 1974b; Dayton and Inturrisi, 1976; Verebey *et al.*, 1976); in man naltrexol has been reported to persist up to 6 days after oral administration (Cone *et al.*, 1974b). In rat the metabolism and excretion of naltrexone are not well defined; less than 1% of the administered dose is found in urine in the first 24 hr (Dayton and Inturrisi, 1976). Essentially no naltrexol is found in dog; naltrexone is conjugated and excreted in the urine (Cone *et al.*, 1974a; Cone and Gorodetzky, 1976). Thus, it appears reasonable to relate the longer duration of naltrexone in man to the persistence of an active metabolite not found in dog. Until the metabolic fate of naltrexone in rat is defined, the long duration of naltrexone remains unexplained in that species.

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