

Pharmacokinetics of Naloxone in Rats and in Man:

Basis for Its Potency and Short Duration of Action

S. H. Ngai, M.D.,* Barry A. Berkowitz, Ph.D.,† J. C. Yang, M.D.,‡
James Hempstead, B.S.,§ Sydney Spector, Ph.D.¶

Using a specific and sensitive radioimmunoassay, naloxone concentrations in the brains and sera of rats were measured at intervals for four hours following iv injection (5 mg/kg). Decrement curves of naloxone were compared with those after iv injection of morphine (5 mg/kg). Serum concentration of naloxone at 5 minutes was $1.45 \pm 0.1 \mu\text{g/ml}$ (mean \pm SE) and that of morphine was $1.0 \pm 0.08 \mu\text{g/ml}$. Their serum half-lives from one to four hours were approximately the same, 30–40 minutes. With naloxone, the brain-serum concentration ratios ranged from 2.7 to 4.6. Concentration of naloxone in the brain declined parallel to that in the serum. However, with morphine the initial brain concentration was approximately one tenth that in the serum ($0.096 \pm 0.04 \mu\text{g/ml}$). The brain morphine concentration was sustained for one hour, while serum morphine concentrations declined from 1.0 to $0.19 \mu\text{g/ml}$ during this period. Two minutes after iv injection of naloxone HCl (0.4 mg) in nine healthy volunteers, the serum drug concentration was $0.01 \pm .001 \mu\text{g/ml}$. At 5 minutes, 97 per cent of the administered dose was no longer found in the serum, the serum concentration being $0.004 \pm .0003 \mu\text{g/ml}$. From 20 minutes to two hours after injection, the calculated mean serum half-life of naloxone was 64 minutes. These results

suggest that the rapid penetration of naloxone into the brain and the high brain-serum concentration ratio contribute to its rapid onset of action and potency as a narcotic antagonist. The rapid decline of naloxone concentration in the brain found in the animal model, in contrast to that of morphine, could be the basis for its relatively short duration of action. (Key words: Pharmacokinetics, morphine; Pharmacokinetics, naloxone; Analgesics, narcotic, morphine; Antagonists, narcotic, naloxone.)

NALOXONE (Narcan), a narcotic antagonist without agonistic action, has become the drug of choice in the treatment of narcotic overdose and in the management of postanesthetic depression induced by narcotics. Several reports have dealt with the relatively short duration of action of this drug.¹⁻³ Assessment by changes in pupillary diameter, respiratory frequency, tidal volumes and minute ventilation indicates that following an intravenous dose of 0.4 mg, naloxone reversed the effects of morphine for approximately 45 minutes.³

We studied the pharmacokinetics of naloxone in rats and in man. Comparison of dispositions of naloxone and narcotics may give the basis for the short duration of action of naloxone.

Methods

Male Sprague-Dawley rats, 250–300 g, received 5 mg/kg of naloxone HCl intravenously. Groups of three to five rats were decapitated 5 and 15 minutes and 1, 2, and 4 hours later. Blood was collected, allowed to clot, and serum frozen until analyzed. The whole brain was removed rapidly, blotted dry, and frozen in dry ice prior to storage and analysis for drug concentration. Similar experiments were done using morphine sulfate, 5 mg/kg, intravenously.

Nine volunteers, five men and four women, 25 to 54 years of age, weighing 57 to 76 kg

* Professor of Anesthesiology and Pharmacology, Columbia University.

† Associate Member, Roche Institute of Molecular Biology.

‡ Assistant Professor of Anesthesiology, Columbia University.

§ Assistant Scientist, Roche Institute of Molecular Biology.

¶ Member, Roche Institute of Molecular Biology; Adjunct Professor of Anesthesiology and Pharmacology, Columbia University.

Received from the Departments of Anesthesiology and Pharmacology, College of Physicians and Surgeons, Columbia University, New York, New York 10032, and Department of Physiological Chemistry, Roche Institute of Molecular Biology, Nutley, New Jersey 07110. Accepted for publication January 13, 1976. Supported in part by Anesthesia Research Center Grant 5P01-GM-09069. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, October 15, 1975.

Address reprint requests to Dr. Ngai.

(mean 63.8 ± 2 , SE), received 0.4 mg of naloxone HCl intravenously. Venous blood samples were obtained from the opposite arm 2, 5, 10, 15, 30, 60, and 120 minutes later and stored in cold until centrifuged. The serum samples were then frozen until assayed.

Detailed procedures of radioimmunoassay for naloxone have been described elsewhere.⁴ Antisera were obtained from rabbits immunized with naloxone-immunogen. The assay is highly sensitive. Two nanograms of naloxone inhibited binding between the antibody and ³H-naloxone by 50 per cent. The antibody recognized the reduced naloxone (EN 2265,** 50 per cent inhibition with 4 ng) but not the principal metabolites in rats and in man.

** Endo Laboratories, Inc.

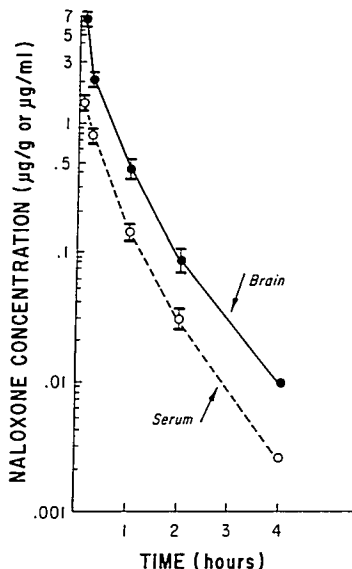


FIG. 1. Serum and brain concentrations of naloxone in rats at intervals following iv injection, 5 mg/kg. Each point represents the mean for 3-5 animals. Vertical bars are SE. Note the higher brain concentrations of naloxone and the parallel decline of drug concentrations with time.

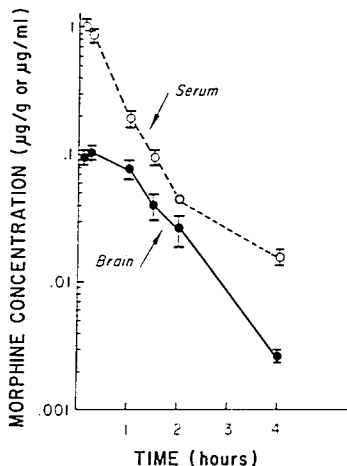


FIG. 2. Serum and brain concentrations of morphine in rats at intervals following iv injection, 5 mg/kg. The brain concentration of morphine was sustained for an hour, while the serum concentration decreased with time.

Assays were carried out using 10-200 µl of serum samples, diluted if the anticipated drug concentration was greater than 1.0 µg/ml, in duplicate. A standard curve of displacement (inhibition) using standard quantities of antiserum, ³H-naloxone and known quantities of naloxone (0.8-6 ng) was prepared for each assay. Brain samples were homogenized with 4 volumes of 0.01 N HCl, and samples of the supernatant were assayed.

Analysis of morphine concentration in serum and brain samples was carried out using rabbit antisera as reported previously.^{5,6} This assay is sensitive enough to measure picogram (10^{-12} g) amounts of free morphine.

Serum half-life of naloxone or morphine was calculated from the decrement curve, using the regression line with least-square fit.

Results

In rats, the serum concentration of naloxone 5 minutes after intravenous injection of naloxone HCl, 5 mg/kg, was 1.45 ± 0.1 µg/ml

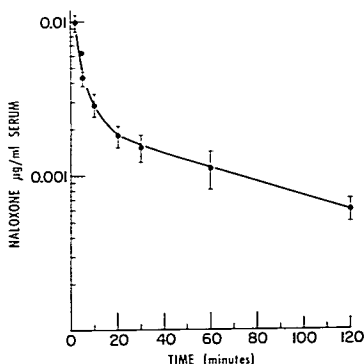


FIG. 3. Mean serum concentrations of naloxone at intervals following iv injection of 0.4 mg in nine subjects. Vertical bars are SEM. The calculated serum half-life is 64 ± 12 minutes (mean \pm SE).

(mean \pm SE). It decreased 0.82 ± 0.08 $\mu\text{g/ml}$ at 15 minutes. From one to four hours after injection the calculated serum half-life was 30 minutes. In the brain, naloxone concentration at 5 minutes was 6.6 ± 0.6 $\mu\text{g/g}$, 4.6 times greater than that in the serum. The decrement curve of brain naloxone concentrations paralleled that for serum. At subsequent intervals, the brain-serum concentration ratios ranged from 2.7 to 3.2 (fig. 1).

Rats that received 5 mg/kg morphine sulfate had a serum concentration of $1.0 \pm .08$ $\mu\text{g/kg}$ at 5 minutes, and a half-life of 40 minutes, similar to that seen following naloxone. However, the brain morphine concentration at 5 minutes was only 0.096 ± 0.04 $\mu\text{g/ml}$, one tenth that in the serum. In addition, the brain concentration of morphine was sustained for one hour, while the serum concentration decreased from 1.0 to 0.19 $\mu\text{g/ml}$ (fig. 2).

In human subjects, 2 minutes after intravenous injection of 0.4 mg naloxone HCl the serum concentration was $0.01 \pm .001$ $\mu\text{g/ml}$. At 5 minutes it was $0.0043 \pm .0003$ $\mu\text{g/ml}$ (fig. 3). Assuming a plasma volume of 3 liters, it may be estimated that the initial serum concentration (at the completion of iv injection) is $400/3000$ or 0.13 $\mu\text{g/ml}$. Thus, at 5 minutes only 3 per cent ($0.0043/0.13$) of the administered dose remained in the circulation.

From 20 to 120 minutes after injection, the mean calculated serum half-life was 64 ± 12 minutes. Individual variations ranged from 30 to 81 minutes.

Discussion

The radioimmunoassay for naloxone used for this study is sensitive and specific. Further, it requires samples of only 10–200 μl . Serum or tissue homogenate may be added directly to the assay system, and a large number of samples may be processed at one time. Using this assay we were able to measure naloxone concentration in human serum in ng/ml range, heretofore difficult to accomplish because of the rather small dose used (less than 1 mg), the tedious extraction procedures involved, and the limited sensitivity of gas-liquid chromatography.⁷ Even with the radioimmunoassay, it was not possible to measure serum naloxone concentration beyond two hours after intravenous injection with confidence.

Comparison of naloxone and morphine concentrations in the serum and brain of the rat might provide some kinetic bases for the relative durations of action of these drugs. Previous studies in rats with naloxone and morphine injected subcutaneously suggested the possibility that the rates of entry into and egress from the brain were important factors in the potency and duration of action of naloxone.⁴ Since differences in absorption following subcutaneous injection could influence the results, dispositions of these drugs are best compared after intravenous administration. In rats, equal intravenous doses of naloxone and morphine gave comparable peak serum drug concentrations and approximately the same serum half-lives. It would seem that in rats there is little difference between the initial rates of elimination of these two drugs (see also Berkowitz *et al.*⁹). However, the brain concentration of morphine, initially one tenth of the serum concentration, was sustained for one hour. In contrast, naloxone entered the brain rapidly. The initial brain concentration of naloxone was much higher (4.6 times) than that in the serum. This could account for the fast onset of action and, in part, the potency of naloxone. Subsequently, naloxone leaves the brain rapidly as the drug is eliminated. Drug con-

centration in the serum reflected that in the brain. This observation could explain the relatively short duration of action of naloxone.

In human subjects, serum decrement curves of naloxone and of morphine are remarkably similar. Re-examination of our previously published data on the disposition of morphine in surgical patients gave mean serum half-lives of 4.8 minutes during the distribution phase (2–10 min), and 62 minutes between 20 and 120 minutes.⁶ The respective mean half-lives of naloxone in nine volunteers were 4.7 and 64 minutes. Therefore, distribution of these drugs in the body as a whole and the rate of elimination could not be the basis for the difference between their durations of action.

Lipid solubilities of naloxone and morphine could account for the marked differences in brain/serum drug concentration ratios of these drugs in rats. Kaufman *et al.* reported that with morphine the distribution coefficient between octanol and water is 1.42, and with naloxone, 33.55 (pH 7.40, 37°C).⁸ However, the initial low brain-serum concentration ratio (0.1) of morphine cannot be explained on the basis of lipid solubility. The possible mechanism(s) for the sustained morphine concentration in the brain are not apparent to us, either.

The serum decrement curve of naloxone in human subjects reported herein does not answer the question concerning the effective serum concentration of naloxone in clinical settings. Johnstone *et al.* used 3.66 µg/kg naloxone intravenously, followed by continuous infusion of 3.66 µg/kg/hr (or 0.26 mg/hr in a 70-kg man) to antagonize the respiratory depressant action of morphine (2 mg/kg).⁹ However, ventilatory response to carbon dioxide returned to control only after four hours of naloxone infusion. During this period, the action of morphine must have been partially dissipated, admittedly to an unknown extent. Perhaps the results of Johnstone *et al.* gave an estimate of the amount of naloxone that has to be infused to maintain a certain serum

(or brain) drug concentration. It is not possible at this time to anticipate how long the action of a given dose of naloxone would last. Narcotics, including morphine, meperidine and fentanyl, each with a different duration of action, are being administered in a wide range of dose levels. With the variable clinical circumstances, our data on the disposition of naloxone reinforce the advice that in cases of narcotic overdose or when large doses of narcotics have been administered, the patient must be closely observed for recurrence of narcotic depression after apparent naloxone-induced reversal.

References

1. Hasbrouck JD: The antagonism of morphine anesthesia by naloxone. *Anesth Analg (Cleve)* 50:954–959, 1971
2. Heisterkamp DV, Cohen PJ: The use of naloxone to antagonize large doses of opiates administered during nitrous oxide anesthesia. *Anesth Analg (Cleve)* 53:12–18, 1974
3. Evans JM, Hogg MIJ, Lunn JN, et al: Degree and duration of reversal by naloxone of effects of morphine in conscious subjects. *Br Med J* 1:589–591, 1974
4. Berkowitz BA, Ngai SH, Hempstead J, et al: Disposition of naloxone: Use of a new radioimmunoassay. *J Pharmacol Exp Ther* 195:499–504, 1975
5. Spector S: Quantitative determination of morphine in serum by radioimmunoassay. *J Pharmacol Exp Ther* 178:253–258, 1971
6. Berkowitz BA, Ngai SH, Yang JC, et al: The disposition of morphine in surgical patients. *Clin Pharmacol Ther* 17:629–635, 1975
7. Weinstein SH, Pfeffer M, Schor JM: Metabolism and pharmacokinetics of naloxone, Narcotic Antagonists. Edited by MC Braude, LS Harris, EL Way, et al. New York, Raven Press, 1973, pp 525–535
8. Kaufman JJ, Semo NM, Koski WS: Microelectrometric titration measurement of the pK_a's and partition and drug distribution coefficients of narcotics and narcotic antagonists and their pH and temperature dependence. *J Med Chem* 18:647–655, 1975
9. Johnstone RE, Jobes DR, Kennell EM, et al: Reversal of morphine anesthesia with naloxone. *ANESTHESIOLOGY* 41:361–367, 1974