Nasal Absorption of Nafarelin Acetate, the Decapeptide [D-Nal(2)^6]LHRH, in Rhesus Monkeys I

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Abstract □ Nafarelin acetate, [D-Nal(2)^6]LHRH, a highly potent superagonist of luteinizing hormone-releasing hormone, was given intranasally to six female rhesus monkeys. Absorption was rapid and very reproducible, with peak levels occurring at 15–30 min and a bioavailability of ~2% relative to a subcutaneous dose. The nasal dose response was highly nonlinear. The nonlinearity was apparently associated with the absorption phase, since elimination profiles at all doses were similar.

Keyphrases □ Nafarelin acetate—nasal absorption, [D-Nal(2)^6]LHRH, rhesus monkeys □ Absorption, nasal—nafarelin acetate, [D-Nal(2)^6]LHRH, rhesus monkeys □ Luteinizing hormone-releasing hormone—analogue, nafarelin acetate, nasal absorption, rhesus monkeys

The delivery of drugs by the nasal route is receiving increased attention as a convenient and efficient method of drug delivery (1–7). In addition to offering advantages such as rapid absorption and avoiding the first-pass effect, it provides for delivery of drugs such as peptides and proteins which are degraded in the GI tract and cannot be given orally. The efficiency of absorption, however, varies considerably between drugs. Whereas a bioavailability of 100% (relative to intravenous) has been reported for nasally administered propranolol in humans (3), the bioavailability was only 1–1.5% for luteinizing hormone-releasing hormone (LHRH) (8). Nevertheless, the high potency of LHRH and its analogues, their large therapeutic ratio, and the need for daily administration in both men and women makes the nasal route extremely attractive for systemic delivery of these compounds.

Nafarelin acetate, a highly potent agonist of LHRH activity, has recently been described (9). In this compound, [D-Nal(2)^6]LHRH, the sixth amino acid in the LHRH decapeptide has been replaced by 3-(2-naphthyl)-D-alanine. This analogue has ~200 times the potency of the native hormone.

The objective of this work was to study the nasal absorption of nafarelin acetate in monkeys, with the purpose of studying the effects of dose and concentration on the systemic availability of this compound *via* nasal administration and establishing a nasal dose equivalent to a therapeutic subcutaneous dose.

EXPERIMENTAL

Study Design—Six adult female rhesus monkeys were used for each dose. The monkeys were sedated before dosing with a 5-mg/kg im injection of ketamine hydrochloride[1]. Approximately 100 μL of a buffered aqueous drug solution was sprayed into each nostril while the animal was held with the head in a vertical position. Immediately after dosing, the animals were placed in a supine position for 5 min.

A heparinized blood sample of ~3 mL was collected from the saphenous vein of each animal at 0 (predose), 5, 15, and 30 min, and 1, 2, 4, and 8 h after administration of the drug. Additional injections of lower doses of ketamine hydrochloride were administered at ~30 min after dosing and immediately prior to the 2-, 4-, and 8-h blood sampling. Blood samples were cooled on ice, and the plasma was separated within 2 h by centrifugation, after which it was stored at −20°C until analyzed by RIA. The monkeys were rested for a minimum of 1 week between doses. In the case of the subcutaneous injection, 0.5 mL of a 10-μg/mL solution of the drug in aqueous buffer was injected in the interscapular area of the monkey, and blood sampling was done as described above.

Radioimmunoassay (10)—A slightly modified nafarelin acetate was conjugated to keyhole limpet hemocyanin (*Megathura crenulta*, mol. wt. 3–7.5 million) using a water-soluble carbodiimide. To provide a suitable functional group for conjugation to the protein, the pyroglutamyl residue in nafarelin acetate was replaced by glutaric acid. Immunization with the protein conjugate was carried out in New Zealand White rabbits using Freund's complete and incomplete adjuvant. The resulting antiserum at a dilution of 1:30,000 yielded a total binding of 45–55%. The radioactive isotope used for labeling was iodine-125. Separation of bound from free radioactivity was carried out with charcoal. The RIA buffer was 0.1 M Tris-HCl, pH 7.2, with 0.5% bovine serum albumin.

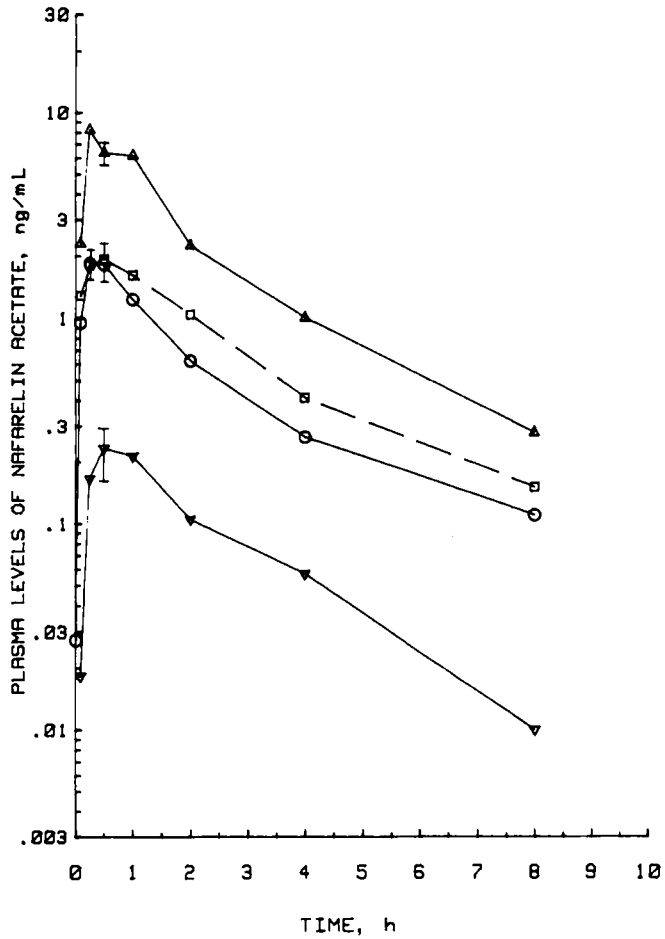

Figure 1—*Mean concentrations (±SEM) of nafarelin acetate in the plasma of monkeys after a single subcutaneous or intranasal dose. Key: (△) 431 μg, nasal; (○) 272 μg, nasal; (▽) 133 μg, nasal; (□) 5 μg, subcutaneous.*

[1] Vetalar; Parke-Davis.

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Table I—Plasma Level Parameters of Nafarelin Acetate Following Nasal and Subcutaneous Administration in Rhesus Monkeys [a]

Conc. of Solution, mg/mL	Average Volume Administered, μL	Average Dose, μg	Peak Height, ng/mL	AUC, ng·h/mL
Nasal				
0.625	213	133(1.8)	0.23(0.07)	0.63(0.3)
1.25[b]	217	272(4.1)	1.84(0.35)	4.56(1.1)
2.5[b]	173	431(15.7)	8.24(0.7)	15.9(2.1)
1.2	121	140(10.3)	0.82(0.28)	2.31(0.76)
Subcutaneous				
0.01	500	5	1.93(0.35)	5.57(0.97)

[a] Six animals per group except where noted; numbers in parentheses are standard errors. [b] Four animals per group due to inadequate dosing of two animals.

The method was validated by adding known quantities of nafarelin acetate to blank plasma and determining the ratios of measured to added compound. Linear regression analysis for the concentration range of 50–500 pg/mL yielded a slope of 0.96 with a correlation coefficient of 0.999. At 50 pg/mL, the *CV* was 11.3%. Additional validation was obtained from an *in vivo* study in which [^{3}H]nafarelin acetate was administered to monkeys, and plasma profiles were determined by RIA and an HPLC–radiochemical method. Excellent agreement was obtained between the two sets of results.

RESULTS AND DISCUSSION

The plasma level profiles of nafarelin acetate for the three nasal doses and the subcutaneous injection are shown in Fig. 1. As with other drugs, nasal absorption is quite rapid, with peak levels being achieved within 15 min. Reproducibility between animals is excellent, particularly at the higher doses, and suggests that at least in a controlled situation, the nasal route has considerable potential for delivery of this LHRH analogue.

The peak heights and areas under the curve (AUC)[2] are listed in Table I. It is immediately evident that there is a pronounced nonlinearity between the dose administered and the resulting levels of drug in the plasma. For example, a roughly twofold increase in dose from 125 to 270 μg results in an approximate 8-fold increase in peak height and a 6.5-fold increase in AUC. Increasing the dose to 430 μg results in a further fourfold increase in peak height and AUC. Since the elimination profiles are similar for all three doses, this suggests that the nonlinearity is associated with changes in the absorption phase. To determine whether the nonlinearity is a function of the concentration of drug in the solution, or of the total dose administered, an additional experiment was conducted where the drug concentration was 1.2 mg/mL (similar to the previous experiment), but the dose was halved by reducing the volume administered. The results shown in Table I suggest that the nonlinearity in peak levels and AUC observed with the increase in dose appears to be associated with the concomitant increase in the concentration of drug in solution. At a concentration of 1.25 mg/mL, a twofold increase in dose results in a slightly greater than twofold increase in peak height. On the other hand, a twofold increase in concentration (from 0.625 to 1.2 mg/mL) results in a 3.5-fold increase in peak height, even though the dose administered is the same (130–140 μg). An increase in flux across the nasal membrane due to the higher concentration or saturable binding and/or metabolism with membrane or enzyme systems are plausible explanations for these observations. Enzymes such as leucine amino peptidase are known to be present in the nasal membrane (11).

In comparing the plasma level profiles for nasal delivery with that found for subcutaneous injection, it is apparent that the plasma levels for the 270-μg nasal dose are almost superimposable on those for the 5-μg sc dose. This gives a bioavailability of ~2% relative to the subcutaneous dose. These data also agree well with bioassay results, where the minimum effective dose for inhibition of ovulation in female rhesus monkeys was 250 and 5 μg administered daily *via* nasal spray and subcutaneous injection, respectively (12). The reason for the relatively low bioavailability of this compound is presently unknown. Enzymatic degradation in the nasal membrane could be a factor, and there may have been some loss of solution *via* postnasal drip. In any case, these studies show that the monkey can serve as a useful animal for studying nasal delivery of drugs.

[2] AUC values were determined for the 0–8-h time period, since plasma blood levels were below detectable limits at 24 h.

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