Effect of Formulation Additives upon the Intranasal Bioavailability of a Peptide Drug: Tetracosactide ($ACTH_{1-24}$)

Patrick Wüthrich,[1,2] Myriam Martenet,[1] and Pierre Buri[1]

Received June 9, 1992; accepted August 18, 1993

Nasal absorption of tetracosactide ($ACTH_{1-24}$; Synacthen) was evaluated in anesthetized rats and compared to intravenous and intramuscular (i.m.) administration. The effect of formulation additives on tetracosactide bioavailability was studied following modification of nasal saline solution. Poloxamer 407 (Pluronic F-127) was used as a vehicle for drug sustained release, whereas sodium glycocholate and bacitracin were used as enhancers. Tetracosactide plasma levels were monitored with radioimmunoassay. Nasal bioavailability was low (4.4%) compared to i.m. (24%). Poloxamer 407 addition did not improve drug kinetics profiles and showed a nonsignificant decrease in bioavailability (4%). On the other hand, both enhancers effectively increased tetracosactide nasal absorption. The sodium glycocholate effect was very fast (T_{max} = 5 min), but did not last long. Moreover, absorption was increased threefold compared to the simple formulation. On the other hand, maximum tetracosactide levels in plasma were reached after 15 min for the formulation containing bacitracin as enhancer, and tetracosactide bioavailability was strongly increased, to 24%, i.e., as much as after an i.m. injection.

KEY WORDS: nasal absorption; peptide drug; tetracosactide; Synacthen; absorption enhancers; bacitracin; sodium glycocholate, poloxamer 407.

INTRODUCTION

Recombinant DNA technology and the development of new peptide synthesis techniques have led to a number of new therapeutic drug candidates available for the clinic (1). However, a major drawback of these compounds is their oral ineffectiveness, mainly because of the digestive proteinase activity of the gut.

If the parenteral route currently represents the most effective way to administer peptide drugs, there are, nonetheless, some problems associated with this administration site, e.g., the short half-life of most peptides requiring frequent injections, the risk of thrombophlebitis, the need for trained health care professionals to administer these compounds to children or elderly people, and the need to have a sterile and pyrogen-free formulation.

Among the alternative routes to parenteral administration of peptide compounds, e.g., rectal, vaginal, and buccal mucosa, the nasal mucosa certainly represents a convenient and reliable route. A highly vascularized mucosa comprising very permeable capillaries, the presence of numerous microvilli creating a large surface area for drug absorption, the absence of an hepatic first-pass effect (2), and the ease of administration are some of the factors that can explain the interest for this administration route.

Moreover, successful nasal delivery of peptide compounds has led to a number of peptide drugs marketed for systemic absorption, such as buserelin (Suprefact, Hoechst), gonadorelin (Kryptocur, Hoechst), protirelin (Relefact TRH nasal, Hoechst), calcitonin (Miacalcic, Sandoz), nafarelin (Synarel, Syntex), and desmopressin (Minirin, Ferring) (3,4).

Although effectively absorbed through the nasal mucosa, the bioavailability of most peptides is low (5). Improved systemic uptake of peptide drugs may be achieved by three strategies: modifications affecting the chemical structure of the peptide candidate to make it more resistant to enzymatic degradation and/or more absorbable, alteration of the absorbtive membrane to increase its permeability coefficient, and addition of suitable additives to protect the peptide against peptidases and/or increase its retention time at the absorption site, thus possibly increasing the bioavailability.

The aim of this work was to study the transnasal transport of a peptide drug, tetracosactide ($ACTH_{1-24}$), after administration to rats and to evaluate the effects of different additives upon its bioavailability, namely, poloxamer 407, sodium glycocholate, and bacitracin. Tetracosactide (Synacthen, Ciba-Geigy) is a linear synthetic polypeptide of molecular weight of 2934 Da. It contains the first 24 amino acids of the natural adrenocorticotropic hormone ACTH (39 amino acids) and possesses the same physiological properties with less antigenicity (6). Nasal bioavailability of tetracosactide was compared to other administration routes, namely, intravenous (i.v.) and intramuscular (i.m.). Poloxamer 407, a block copolymer of polyoxyethylene and polyoxypropylene, was evaluated as a vehicle for drug sustained release. This compound possesses interesting reversible thermogelling properties and has shown bioadhesive properties (7). Sodium glycocholate has been extensively studied as a penetration enhancer for nasal delivery of peptide drugs such as insulin (8) or nafarelin (9). Bacitracin permeation enhancing properties have been demonstrated for luteinizing hormone releasing hormone (LHRH) and buserelin, thus making it an attractive candidate for the enhancement of peptide drugs (10).

MATERIALS AND METHODS

Solution Preparation

The solutions for parenteral administration, i.v. and i.m., were prepared after appropriate dilution (v/v) of Synacthen bottles, 0.25 mg/mL (Ciba-Geigy, Basel, Switzerland), with a 0.9% saline solution.

The solutions for intranasal (i.n.) administration consisted of (i) a saline solution used as a control; (ii) tetracosactide acetate (Ciba-Geigy) solution, 0.62 mg/mL (w/v), in saline; (iii) tetracosactide acetate solution, 0.61 mg/mL (w/

[1] School of Pharmacy, University of Geneva, 30 quai E. Ansermet, 1211 Geneva 4, Switzerland.

[2] To whom correspondence should be addressed at EUROPEPTIDES, Bt Aristote, 9 Av. du Marais, 95100 Argenteuil Cedex, France.

0724-8741/94/0200-0278$07.00/0

v), in saline with 20% (w/v) poloxamer 407 (Pluronic F-127, BASF Schweiz, Wädenswill-Switzerland); (iv) tetracosactide acetate solution, 0.39 mg/mL (w/v), in saline with 1 % (w/v) sodium glycocholate (Sigma, St. Louis, MO); and (v) tetracosactide acetate solution, 0.63 mg/mL (w/v), in saline with 0.01 *M* bacitracin (Fluka, Buchs, Switzerland).

Animal Preparation

Male rats Zur:SIV f(SPF) weighing 290–390 g were used after 1 week of acclimation to animal husbandry conditions. Three hours prior to anesthesia, the animals received a subcutaneous injection of a dexamethasone sodium phosphate solution (Decadron phosphate ophthalmic drops, MSD, Zürich, Switzerland) at 1 mg/kg calculated as dexamethasone base. The aim of this procedure was to block endogenous ACTH secretion to prevent ACTH from interfering during the analytical procedure of tetracosactide (6,11). The animals were then anesthetized with an injection of sodium pentobarbital i.p. 50 mg/kg (Vetanarcol, Veterinaria, Zürich, Switzerland) and prepared for nasal administration using a surgical procedure adapted from Hirai *et al.* (12). To prevent the instilled solution from being eliminated through the esophagus, the latter was tied off with a suture. During the course of the experiments, the animals were kept under a heat lamp to maintain a normal body temperature.

The doses administered were chosen according to the literature to achieve drug levels in the plasma sufficiently high to be determined with our analytical method; i.v., 6.66 μg/kg in the jugular vein; i.m., 8.33 μg/kg in the thigh muscle; and i.n., 100 μg/kg into one nostril for the preparations without additive as well as those containing poloxamer 407 and bacitracin. For the preparation containing sodium glycocholate, the dose was 67 μg/kg. The injection volume for the parenteral administrations was 200 μL/300 g.

The nasal administration was performed with polypropylene tubing (PP-20, Portex, G.-B.) connected to an 100-μl Hamilton syringe (Bonaduz, Bonaduz, Switzerland). The volume instilled was 50 μL/300 g into one nostril.

One hundred microliters of blood was sampled through a catheter (Silastic, Dow Corning, Detroit, MI) inserted into the jugular vein. A syringe filled with saline was connected to the catheter to prevent clot formation and it was used for injecting saline to maintain the volume of body fluid approximately constant. Blood was collected into tubes chilled in ice and containing a preserving mixture of EDTA (Merck, Darmstadt, Germany), aprotinine (Trasylol, Fluka), and mercaptoethanol (Fluka). Under these conditions tetracosactide degradation was kept minimal (13,14).

The blood was immediately centrifuged at 4°C, and the plasma separated and kept frozen at −18°C until subsequent analysis.

Analytical Procedure

The drug levels in the plasma were monitored with radioimmunoassay (RIA) (ACTH RIA-100, Medgenix, Fleurus, Belgium) on 50-μL plasma samples. Scintillation counting was performed on an LKB 261 (LKB, Bromma, Sweden) Multigamma counter.

Calculations

The area under the curve of plasmatic concentrations between 0 and 120 min (AUC_{0-120}) was calculated with the trapezoidal rule. Bioavailability (F) was calculated according to the following formula:

$$F(\%) = \frac{\overline{(AUC)}_x \times \bar{d}_{i.v.}}{\overline{(AUC)}_{i.v.} \times \bar{d}_x} \times 100$$

where $\overline{(AUC)}$ represents the mean value of individual data calculated and $\bar{d}$ represents the mean value of individual administered doses. The subscript i.v. is for the intravenous injection and x for the tested route. The values for plasma concentrations after i.v. administration at time 0 were extrapolated by logarithmic linear regression.

The mean control plasma concentration–time curve was chosen as the baseline describing endogenous ACTH secretion during the course of the experiment. Any individual value for i.v., i.m., and i.n. administration found below the baseline was discarded and then replaced with the mean value of the baseline.

RESULTS

Route of Administration

Figure 1 shows the mean tetracosactide concentrations in the plasma after i.v., i.m., and i.n. administration. The nasal administration of a saline solution used as a control resulted in very low plasma ACTH levels, thus indicating an effective blockade of the endogenous ACTH secretion by the steroid given prior to the tetracosactide administration.

Tetracosactide kinetics in the plasma after i.v. injection show a biphasic decay. One can calculate an elimination half-life ($t_{1/2}$) of 3.7 min for the first phase (0–20 min) and 45 min for the second phase (20–120 min). Intramuscular administration led to much lower drug levels, even though the doses injected were similar to those of the i.v. injection. The drug concentration in plasma (C_{max}) reached maximum after 4 min (T_{max}).

Tetracosactide was absorbed through nasal mucosa fol-

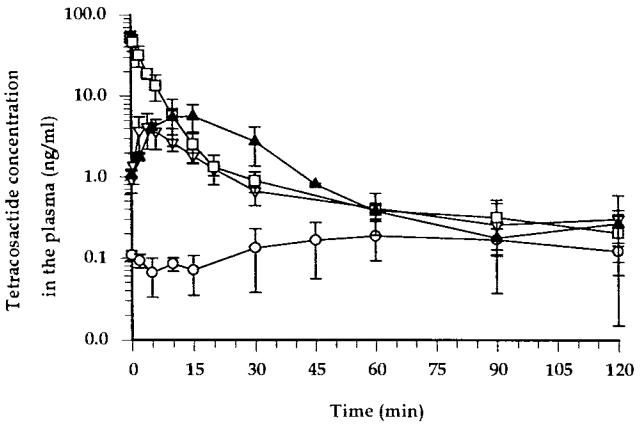

Fig. 1. Tetracosactide levels in the plasma after administration by different routes in rats. Tetracosactide: (□) 2.43 μg/rat i.v.; (▽) 2.96 μg/rat i.m.; (▲) 33.47 μg/rat i.n. (○) Saline solution i.n. Each point represents the mean ± SD for three animals.

lowing the instillation of a simple saline solution of the peptide. The T_{max} occurred at 10–15 min, substantially later than after i.m. administration.

Table I presents the AUC obtained after the administration of the drug to different routes. The nasal bioavailability, as shown in Fig. 2, is low compared to that with the i.v. injection (4.4%). This result is not surprising considering the size and the nature of the drug. The bioavailability after i.m. injection reached 24%.

Effect of Poloxamer

To modify the drug release and consequently the pharmacokinetic parameters, poloxamer 407 was incorporated into a nasal formulation. As shown in Fig. 3., plasma concentrations of tetracosactide were only marginally lower than without additive (not significant). The T_{max} also occurred at 10 min and the bioavailability (Fig. 2) was similar to that of tetracosactide alone.

Effect of the Enhancers

Two penetration enhancers added to the nasal formulation both dramatically increased tetracosactide plasma levels (Fig. 4). Sodium glycocholate promoted a rapid penetration of the peptide into the bloodstream, as evidenced by a T_{max} of only 5 min. This value was similar to that obtained after i.m. injection. In addition, plasma drug levels returned to the baseline after 30 min. In comparison, the T_{max} of the formulation containing bacitracin was delayed and appeared 10 min later. Moreover, a broader plasma curve was observed, with drug levels returning to the baseline after 90 min. Table II summarizes the AUC obtained with the two enhancers used during this study. Bacitracin increases the bioavailability of the drug to 24%, whereas with sodium glycocholate a value of 12% was reached (Fig. 2).

DISCUSSION

As early as 1952, according to McKendry *et al.* (15), Paulson *et al.* demonstrated that ACTH could be absorbed through the nasal mucosa. Jeffcoate *et al.* (16) compared the efficacy of two ACTH analogues, namely, $ACTH_{1-24}$ and $ACTH_{1-18}$, given to human volunteers.

In our study, nasal administration of tetracosactide resulted in a low bioavailability but remained in the range of values found for peptides of the same molecular weight. For example, secretin, a linear peptide of molecular weight 3052 Da, was found to be absorbed to the extent of 4% (17).

To modify the transnasal kinetic profiles of tetracosactide, poloxamer 407 was evaluated as an excipient because

Table I. Mean Area Under the Curve (AUC) Between 0 and 120 min Following Different Administration Routes

Administration route	Dose ± SD (μg)	AUC_{0-120} ± SD (ng · min/mL)
Control	—	17.3 ± 6.9
i.v.	2.43 ± 0.21	288.9 ± 81.2
i.m.	2.96 ± 0.14	96.1 ± 30.7
i.n.	33.47 ± 5.40	180.9 ± 53.5

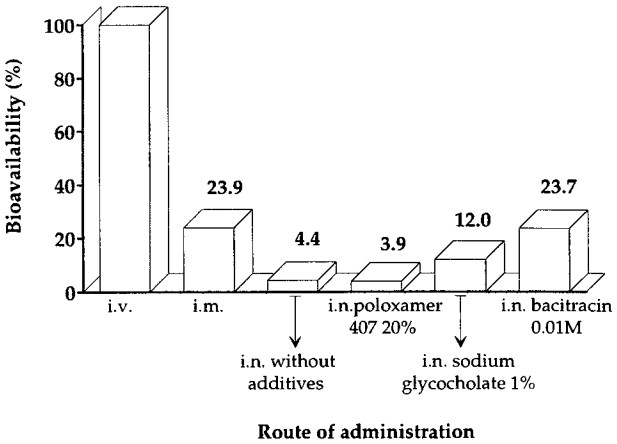

Fig. 2. Effect of administration route and formulation additives on tetracosactide bioavailability in rats. The numbers above each column represent the bioavailability in relation to i.v. administration.

of its low toxicity in topical and oral applications (18, 19). Poloxamer 407 is a nonionic surfactant and thus may present some enhancing properties as demonstrated for poxyethylene 9 lauryl ether. In addition, Juhasz *et al.* (20) have shown that the diffusion coefficient of rat 1–28 atrial natriuretic peptide (ANF; MW 3060 Da) was significantly reduced in poloxamer 407 gels at 37°C *in vitro*. However, in our *in vivo* model no such slow release was observed, indicating that the *in vitro* test does not predict the *in vivo* outcome where the gel is spread at an uncontrolled thickness directly on an aqueous medium. The slight decrease in the plasma concentrations might suggest some interaction of the peptide with poloxamer. It might also suggest that the sustained-release effect of poloxamer can be detrimental to the absorption of the peptide; this could be explained by the slow release kinetics of the peptide at the absorption site, where it would be immediately and extensively metabolized by the peptidases. Such a detrimental effect has been reported for GhRP-6 (six amino acids growth hormone releasing peptide) formulations containing methylcellulose as additive; in this case a 50% decrease in AUC was observed (21).

In the last part of this study, two chemically different

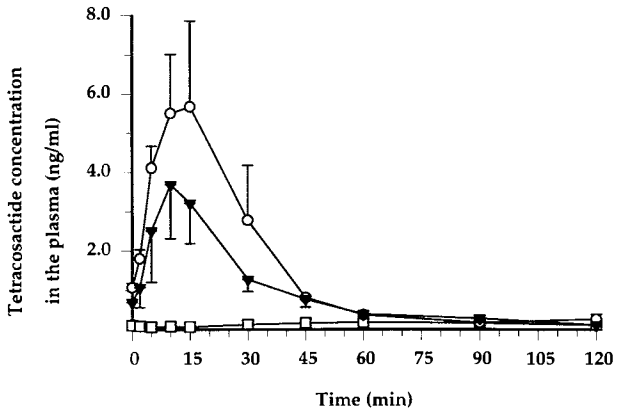

Fig. 3. Effect of poloxamer 407 addition on nasal absorption of tetracosactide in rats. Tetracosactide: (○) 33.47 μg/rat; (▼) 29.59 μg/rat with 20% poloxamer 407. (□) Saline solution. Each point represents the mean ± SD for three animals.

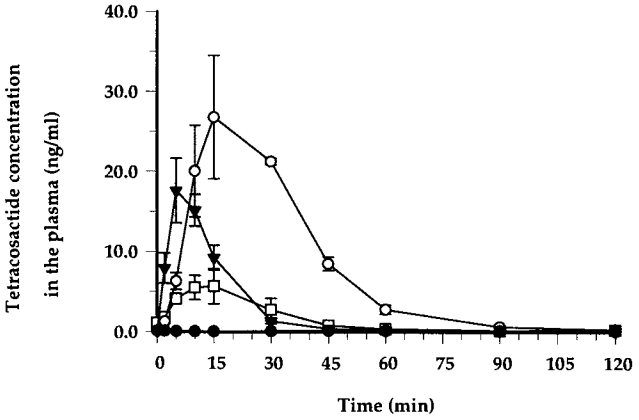

Fig. 4. Effect of enhancer type on nasal absorption of tetracosactide in rats. Tetracosactide: (□) 33.47 μg/rat; (▼) 20.87 μg/rat with 1% sodium glycocholate; (○) 34.41 μg/rat with 10^{-2} *M* bacitracin. (●) Saline solution. Each point represents the mean ± SD for three animals.

enhancers were added to tetracosactide formulations, namely, sodium glycocholate and bacitracin. The results, as shown in Fig. 3, clearly demonstrate the efficacy of both compounds.

Sodium glycocholate causes rapid absorption of tetracosactide through the nasal mucosa. The T_{max} resulting from the intramuscular administration is equivalent to that of the intranasal administration with sodium glycocholate. This interesting enhancing effect of sodium glycocholate might be particularly useful when an immediate pharmacological effect is desired. Among the mechanism(s) of action of bile salts (22), their damaging effect upon mucosal membranes (23) is of concern if such compounds are to be used in the clinic. Studies on the potential of mucosal membranes to regenerate following repeated applications of bile salts, such as sodium glycocholate, are needed before conclusions can be drawn as to their overall toxicity.

On the other hand, with bacitracin, tetracosactide maximum concentration levels in the plasma were reached only after 15 min, thus suggesting a different mechanism of action compared to sodium glycocholate. Moreover, bacitracin caused a sixfold increase in tetracosactide bioavailability compared to the formulation without additives and a twofold increase when sodium glycocholate was used (Fig. 2). This remarkable effect is not yet fully understood, but Raehs *et al.* (10) made similar observations following the transnasal absorption of buserelin and LHRH formulations containing bacitracin as enhancer. Bacitracin's only known property that could explain its activity as an enhancer is its aminopeptidase inhibiting activity *in vitro*. Bacitracin is currently used to inhibit gonadotropin releasing hormone (GnRH) agonist buserelin degradation in the plasma (24). Raehs (25) studied bacitracin as an enhancer for nasal delivery of peptide drugs such as buserelin, calcitonin, and growth releasing factor (GRF). The authors demonstrated that the potency of bacitracin is variable depending on the manufacturer, bacitracin from Calbiochem (California, USA) being more potent than bacitracin from Sigma (München, Germany). Further, bacitracin A alone, a major component of bacitracin, was not responsible for the enhancing properties. Finally, it was shown that the enhancing effect of bacitracin was not due to an irreversible damage to the mucosa.

Our findings indicate that bacitracin is more potent than sodium glycocholate for the delivery of tetracosactide. Therefore bacitracin might be preferred over bile salts and related compounds as a penetration enhancer for the nasal delivery of peptides.

Table II. Effect of Formulation Composition on the Intranasal Mean Area Under the Curve (AUC) Between 0 and 120 min

Formulation type	Dose ± SD (μg)	AUC_{0-120} ± SD (ng · min/mL)
Control	—	17.3 ± 6.9
i.n. without additives	33.47 ± 5.40	180.9 ± 53.5
i.n. with poloxamer 407, 20%	29.59 ± 0.94	145.2 ± 12.7
i.n. with sodium glycocholate, 1%	20.87 ± 1.18	296.2 ± 44.2
i.n. with bacitracin, 0.01 *M*	34.41 ± 1.09	929.1 ± 109.1

ACKNOWLEDGMENTS

The authors would like to thank Dr. Paul Bishof of Geneva's Cantonal Hospital, Department of Obstetric and Gynaecology, for access to the equipment for determining the tetracosactide concentration in the plasma. Ciba-Geigy's (Basel, Switzerland) kind gift of Synacthen and tetracosactide was greatly appreciated by the authors. Professor V. Lenaerts is gratefully acknowledged for valuable advice and comments.

REFERENCES

1. P. D. Gesellchen and R. F. Santerre. Synthesis of peptides and proteins by chemical and biotechnological means. In V. H. L. Lee (ed.), *Peptide and Protein Drug Delivery*, Marcel Dekker, New York, 1991, pp. 57–135.
2. P. Wüthrich and P. Buri. Doelker. Aspects de l'anatomie et de la physiologie nasale. *Pharm. Acta Helv.* 64:322–331 (1989).
3. E. Doelker. Innover pour réussir. *J. Suisse Pharm.* 18:490–495 (1991).
4. A. S. Harris. Biopharmaceutical aspects on the intranasal administration of peptides. In S. S. Davis, L. Illum, and E. Tomlinson (eds.), *Delivery Systems for Peptide Drugs*, Plenum Press, New York, 1986, pp. 191–204.
5. C. McMartin, L. E. F. Hutchinson, R. Hyde, and G. E. Peters. Analysis of structural requirement for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.* 76:535–540 (1987).
6. R. C. Gaillard. Régulateurs de la fonction hypothalamo-hypophysaire. In M. Schorderet (ed.), *Pharmacologie, Des concepts fondamentaux aux applications thérapeutiques*, Slatkine, Genève, 1988, pp. 433–436.
7. J. Juhasz, C. Pimienta, and V. Lenaerts. Adhesion of poloxamer 407 formulations on dog ileal segments in vitro. *Eur. J. Pharm. Biopharm.* 37:262–265 (1991).
8. Y. W. Chien and S. F. Chang. Historic development of transnasal systemic medications. In Y. W. Chien (ed.), *Transnasal Systemic Medications*, Elsevier, Amsterdam, 1985, pp. 1–99.
9. S. T. Anik and M. Johnson. Preformulation and formulation considerations of peptide drugs: Case history of an LHRH analog. In V. H. L. Lee (ed.), *Peptide and Protein Drug Delivery*, Marcel Dekker, New York, 1991, pp. 57–135.
10. S. C. Raehs, J. Sandow, K. Wirth, and H. P. Merkle. The ad-

juvant effect of bacitracin on nasal absorption of gonadorelin and buserelin in rats. *Pharm. Res.* 5:689–693 (1988).
11. M. Normand, J. Lalonde, and N. Barden. Studies and dynamics of adrenocortical responses to ACTH in the rat. *Mol. Cell Endocrinol.* 27:97–106 (1982).
12. S. Hirai, Y. Yashiki, T. Matsuzawa, and H. Mima. Absorption of drugs from the nasal mucosa of rat. *Int. J. Pharm.* 7:317–325 (1981).
13. *Users Guide for ACTH-RIA-100® Detection Kit,* Medgenix, Belgium, 1986.
14. G. M. Besser, D. N. Orth, W. E. Nicholson, R. L. Byyny, K. Abe, and J. P. Woodham. Dissociation of the disappearance of bioactive and radioimmunoactive ACTH from plasma in man. *J. Clin. Endocrinol.* 32:595–603 (1971).
15. J. B. R. McKendry, H. Schwartz, and M. Hall. Intranasal corticotropin—its physiological and clinical effects. *Can. Med. Assoc. J.* 70:244–248 (1954).
16. W. J. Jeffcoate, C. Phenekos, J. G. Ratcliffe, S. Williams, L. Rees, and G. M. Besser. Comparison of the pharmacokinetics in man of two synthetic ACTH analogues: Alpha$^{1-24}$ and substituted alpha$^{1-18}$ ACTH. *J. Clin. Endocrinol.* 7:1–11 (1977).
17. T. Ohwaki, H. Ando S. Watanabe, and Y. Miyake. Nasal absorption of secretin in rats. In M. H. Rubinsten (ed.), *Pharmaceutical Technology. Controlled Drug Release, Vol. 1,* Ellis Horwood, Chichester, 1987, pp. 72–81.
18. K. Ebbens, C. Mastri, and M. Keplinger. Technical Bulletin, *Pluronic Polyols Toxicity and Irritation Data,* 3rd ed., rev., BASF Wyandotte Corp., 1973.
19. J. E. F. Reynolds. *Martindale. The Extra Pharmacopoeia,* 29th ed., Pharmaceutical Press, London, 1989.
20. J. Juhasz, V. Lenaerts, P. Raymond, and H. Ong. Diffusion of rat atrial natriuretic factor in thermoreversible poloxamer gels. *Biomaterials* 10:265–268 (1989).
21. Ch. K. Oh, G. A. Christensen, M. J. Reginato, R. A. Macia, and J. K. Vora. Effect of intranasal formulation variables on pharmacologic availability of growth hormone releasing peptide. Abstract book of the 2nd European Symposium on Controlled Drug Delivery, Noordwijk aan Zee, 1992, pp. 75–76.
22. R. DePoni and E. Lardini, Use of chemical enhancers for nasal drug delivery *Drug Dev. Ind. Pharm.* 17:1419–1436 (1991).
23. G. P. Martin, L. M. El-Hariri, and C. Marroitt. Bile salt- and lysophosphatidylcholine-induced membrane damage in human erythrocytes. *J. Pharm. Pharmacol.* 44:646–650 (1992).
24. H. M. Behre, J. Sandow, and E. Nieschlag. Pharmacokinetics of the gonadotropin-releasing hormone agonist buserelin after injection of a slow-release preparation in normal men. *Arzneim. Forsh./Drug Res.* 42:80–84 (1992).
25. S. C. Raehs. *Cyclopeptide zur Förderung der nasalen Absorption von Peptidarzneistoffen,* Ph.D. thesis, Bonn, 1989.