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EDITORIAL

SYMPOSIUM ARTICLES

ARTICLES

Contents continued on III

Contents continued from I

BOOK REVIEWS

Intranasal Administration of Peptides: Nasal Deposition, Biological Response, and Absorption of Desmopressin

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Received June 9, 1986, from the *Faculty of Pharmacy, Biomedical Centre, S-751 23 Uppsala, §Department of Coagulation Disorders, Malmö General Hospital, S-214 01 Malmö, ‡Fyzikon AB, S-221 00 Lund, and the ¶Department of Pharmacology, Ferring Pharmaceuticals, S-200 62 Malmö, Sweden. Accepted for publication September 2, 1986. Present address: △Ferring Research, S-200 62 Malmö, Sweden.

Abstract □ The nasal administration of desmopressin [1-(3-mercaptopropionic acid)-8-D-arginine-vasopressin] in humans was investigated. Desmopressin solutions containing ^{99m}Tc-labeled human serum albumin were administered intranasally as a spray, using two metered-dose pumps, or as drops, using a rhinyle catheter or a single-dose pipet. Images of the sites of deposition and rates of clearance were monitored quantitatively by gamma scintigraphy. Plasma levels of desmopressin were measured using a highly sensitive and specific radioimmunoassay. The biological response was determined by measuring circulating levels of F VIII, the antihemophilia factor. The sprays were deposited mainly anteriorly, from which small portions were cleared slowly into the nasal pharynx. In contrast, the drops were deposited mostly posteriorly and cleared very rapidly in large portions; some were swallowed immediately. Plasma levels showed that desmopressin was absorbed to a greater extent after administration of the spray with a 2 to 3-fold increase in the relative bioavailability compared with the drops. The biological response was clearly enhanced after spray administration and produced similar increases in F VIII activity. A linear correlation was observed between maximum plasma desmopressin levels and maximum F VIII activity. The use of an intranasal spray device can deposit well-controlled doses within the nasal cavity, which remain there sufficiently long to provide a clear enhancement in absorption and bioavailability.

It has recently become evident that intranasal administration is useful for delivering drugs to the systemic circulation. In the past, the administration of drugs by the nasal route has concentrated on local action on the mucosa. The large surface area of the nasal cavity, its highly vascularized bed of mucosa, and the fact that it appears to contain little metabolizing capacity,[1] all contribute toward making it a useful site for drug absorption. Drugs which have hitherto only been administered parenterally have become the primary candidates. In particular, polypeptides, such as insulin,[2] luteinizing hormone releasing hormone (LHRH),[3] secretin,[4] and growth releasing factor (GRF),[5] have been investigated for their therapeutic activity following intranasal administration.

The standard method of administration has been in the form of sprays or drops which have been delivered using rhinyle catheters, single-dose pipets, or metered-dose, precompression spray pumps. It has been postulated that the site of deposition and rate of clearance of the drug will influence its absorption and, therefore, the therapeutic effect.[6] Moreover, it has been demonstrated that the choice of the delivery system, whether by spray or drops, will influence the site of deposition and its subsequent clearance.[6,7,8] A study by Hardy et al.[6] compared the deposition and clearance of solutions administered by spray (100 μL) and by one (30 μL) or three nasal drops (90 μL). The nasal spray was found to deposit anteriorly in the nasal atrium in contrast to the drops which dispersed throughout the length of the nasal cavity. As a consequence, the spray was found to clear more slowly than the drops, since most of the spray deposited on nonciliated regions.

Although these previous studies have investigated in some detail the method and technique of nasal administration, no work has been done on the relationship between deposition and in vivo absorption and the effect of nasal delivery systems on resulting biological response. Using the peptide desmopressin (DDAVP) as a model, we compared various methods of intranasal delivery by measuring the deposition and clearance of drugs in the nasal cavity using gamma scintigraphy and systemic absorption using a specific radioimmunoassay, and, by determining the effect on circulating levels of the antihemophelia factor (F VIII).

Experimental Section

Materials—Desmopressin [1-(3-mercaptopropionic acid)-8-D-arginine vasopressin; Minirin, lot no. 85K24, Ferring Pharmaceuticals, Malmö, Sweden] and ^{99m}Tc-labeled human serum albumin (TechneScan, Microspheres 20/40, Mallinckrodt Diagnostica, Holland) were used. All reagents were analytical grade.

Nasal Formulations—The desmopressin (DDAVP) solutions were prepared under aseptic conditions. Nasal formulations were prepared containing either 1.5 or 3.0 mg/mL desmopressin in 0.9% sodium chloride (w/v) and 5% chlorobutanol (w/v).

The rhinyle delivery device was a calibrated plastic catheter designed to give a dose of 200 μL or 300 μg from a solution of 1.5 mg/mL desmopressin. The intranasal single-dose pipet was supplied in the commercial form, manufactured using the "Bottelpack" (Rommerlag GmbH, Switzerland) principle of fill, form, and seal in a single operation. Each pipet contained 200 μL or 300 μg of 1.15 mg/mL desmopressin. Nasal sprays were supplied as precompression, metered dose, spray pumps (Pfeiffer GmbH, Radolfzell, West Germany). Two pumps were tested: one gave a volume of 100 μL or 150 μg of 1.5 mg/mL desmopressin; the other gave a volume of 50 μL or 150 μg of 3.0 mg/mL drug per actuation. Radiolabeling of each device was done by addition of 1 mg of human serum albumin radiolabeled with 100–200 MBq ^{99m}Tc. Each dose contained 2–4 MBq ^{99m}Tc.

Deposition Studies—The nasal solutions were administered to six healthy male volunteers aged 30 years or more. None of the subjects had nasal problems and all were free from colds. Separated by an interval of at least 3 d, each subject received all four treatments which were allocated in a blind, randomized sequence using coded, sealed envelopes. In this way, a total of 24 administrations were made. The study was approved by the hospital ethical committee and radioisotope committee, and each subject gave written informed consent prior to entry into the study.

All nasal solutions were administered with the subjects sitting in an upright position and into the same nostril on each occasion. A standard dose of 300 μg of desmopressin was self-administered in every case. The rhinyle drops were administered by first filling 0.2 mL of solution into the tube. One end of the tube was then put into the mouth, the other end was introduced 5–10 mm into the nostril, and delivery was accomplished by blowing. The dose from the pipet was administered by the following procedure: the subject's head was tilted back and individual drops were dispensed into the nostril during normal breathing. The head was turned to the right and left and then back to the original position before the subject assumed a normal sitting position. The two nasal sprays were designed to give accurate doses of 50 and 100 μL, respectively. Prior to administration, each spray device was primed by activating the pump five

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times. The applicator tip was introduced 5 to 10 mm into the nostril, and two doses of 100 or 50 μL were dispensed during normal inhalation, with the contralateral nostril open. Throughout the study, and at each monitoring period, the subjects continued to breath normally but did not blow their noses or sneeze. Food and drink were withheld for the first hour and thereafter were allowed ad libitum.

At the instance of dosing, each subject was seated adjacent to the gamma camera head. Immediately after dosing, the tracer was monitored repeatedly by the gamma camera, and a lateral view of the head was recorded for 10 min. Additional images were recorded at 15-min intervals for the first hour and then at 1- or 2-h intervals up to 8 h.

The data were recorded by computer (PDP (11/34), Gamma 11 V3.1) for subsequent analysis. Each image was displayed on a monitor, and regions of interest were demarcated. The count rate at each monitoring period was corrected for radioactive decay. Each count rate in each region of interest was expressed as a percentage of the initial count rate taken immediately after dosing.

Blood Collection—Blood samples were collected by venipuncture (21-gauge needles) at times 0, 15, 30, 45, and 60 min and 2, 4, and 8 h after administration. Blood was collected in a 3.8% trisodium citrate solution at a ratio of 9 to 1. Platelet-poor plasma was obtained after centrifugation at 2000 × *g* for 10 min at 4 °C, and stored frozen at −50 °C until assayed.

Assay Methods—Plasma desmopressin (DDAVP) was assayed using a sensitive and specific radioimmunoassay (RIA). Antiserum to desmopressin (DDAVP) was developed as described by Sofroniew et al.[9] Since desmopressin (DDAVP) lacks the N-terminal amino group, it was necessary to use 8-D-arginine vasopressin (8-D-AVP), which was coupled to thyroglobulin.[10] The monoiodinated derivative of desmopressin (DDAVP) was prepared by the chloramine T method.[11] Briefly, 5 μmol of desmopressin (DDAVP, 0.25 mg/mL in 0.05 M sodium phosphate buffer, pH 7.5) was added to 1 mCi of $Na^{125}I$ (IMS 30, Amersham) and incubated for 60 s. The monoiondinated tracer was immediately isolated by reversed-phase HPLC (27% acetonitrile in 0.1% trifluoroacetic acid) giving a specific activity of at least 1600 mCi/μmol. The RIA contained 100 μL of standard (5.0–640 pg/mL) or sample diluted five times with assay buffer, 100 μL of tracer (~5000 cpm), and 200 μL of antiserum (1:100 000). A 100-μL aliquot of normal human serum, diluted 5-fold, was added to the standard and 100 μL of assay buffer was added to the sample tubes. Incubations were carried out for 48 h at 4 °C, followed by separation of bound and free radioactivity by addition of 1 mL of plasma-coated charcoal. The assay diluent was 0.05 M sodium phosphate buffer, pH 7.5, containing 0.01% sodium acetate and 0.1% human serum albumin. The F VIII coagulant activity (F VIII:C) was measured by chromogenic substrate assay (Coatest, KabiVitrum, Stockholm, Sweden) according to Rosen et al.[12] The F VIII/von Willebrand factor (F vWF) was measured by immunoradiometric assay (IRMA) according to Ruggeri et al.[13] To eliminate the large systematic differences observed between subjects, the samples obtained before administration were given an arbitrary potency of 100% for F VIII:C and F vWF.

Statistical Methods—Friedman's two-way analysis of variance by ranks and the Wilcoxon matched-pairs, signed rank test were used where appropriate. In addition t_{max}, C_{max}, and $t_{1/2}$ were calculated for plasma profiles of desmopressin after each method of administration. The AUC was determined, using the logarithmic trapezoidal rule, from the plasma desmopressin concentration versus time curve. Linear regression was calculated using the least-squares method.

Results and Discussion

Deposition Studies—Analysis of the data revealed a distinct difference in the pattern of deposition and clearance between the nasal sprays and nasal drops. The pipet and rhinyle tube appeared to deposit solution toward the rear of the nasal cavity at the site of the nasopharynx, while the nasal sprays tended to deposit anteriorly in the nasal cavity (Fig. 1). As the images were taken continually for the first 10 min, it was possible to visualize the dynamic clearance of each solution from its site of deposition. It was apparent that the major amount of drug administered by the pipet and rhinyle tube was cleared very rapidly, while the drops deposited by the sprays tended to break up into smaller

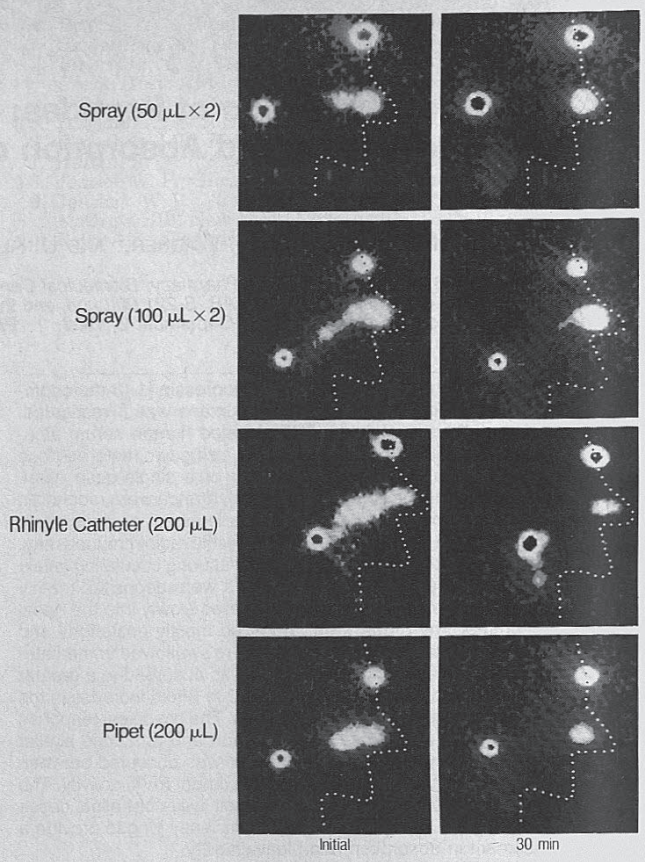

Figure 1—*Gamma scintigraphy images of sites of deposition and patterns of clearance following administration of sprays by two nasal pumps and drops by rhinyle catheter and pipet. Each pair of images is from the same subject, however, the four pairs are from different subjects.*

droplets. The latter were cleared at a slower rate, as ciliary movement transported these droplets back to the nasopharynx. These qualitative findings were complimented by qualitative data on the relative rate of clearance of each solution. The curves revealed a biphasic pattern of clearance; a fast initial clearance followed by a slower, more prolonged second phase (Fig. 2). It was the initial, fast phase that clearly distinguished the nasal drops administered by the pipet and rhinyle catheter from the spray drops. The former showed a 50% clearance within 14 and 20 min for the pipet and rhinyle catheter respectively in contrast to 120 and 240 min for the 200- and 100-μL sprays, respectively. These results are in agreement with the findings of Aoki and Crawley[8] who showed that drops were cleared faster than sprays. However, in contrast, our data shows the rate of clearance to be much faster for all solutions, but specifically so for the nasal drops. Indeed, many subjects commented subjectively on a sensation of swallowing the drops when given by the pipet and rhinyle catheter, as they were rapidly cleared in large portions into the throat. These differences may be due to the method of delivery, as our subjects were sitting upright in contrast to the supine position described by Aoki and Crawley.[8]

Plasma Profile—The plasma profile of desmopressin after each method of administration is shown in Fig. 3. The results show a clear distinction in plasma levels between the nasal sprays and nasal drops. Peak plasma levels were significantly greater after nasal sprays than after nasal drops ($p <$

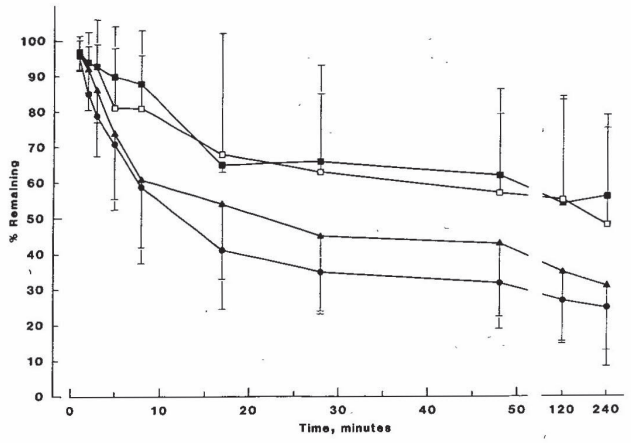

Figure 2—*Clearance of the ^{99m}Tc-radiolabeled tracer from the nasal cavity after administration of 100 μL of spray (■—■), 200 μL of spray (□—□), and drops by a rhinyle catheter (●—●), and pipet (▲—▲), each to six subjects (mean ± SEM).*

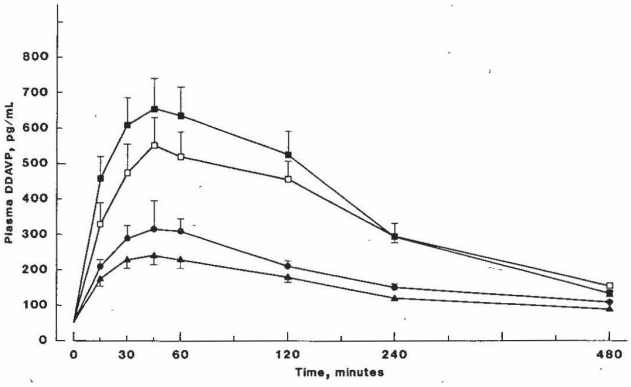

Figure 3—*Plasma levels of desmopressin following nasal administration of 300 μg of desmopressin in 100 μL of spray (■—■), 200 μL of spray (□—□), and drops by a rhinyle catheter (●—●), and pipet (▲—▲) (mean ± SEM).*

0.001). The pharmacokinetic data (Table I) show a significantly greater AUC after nasal spray administration than after nasal drops ($p < 0.001$), indicating a 2- to 3-fold greater bioavailability. No significant difference in plasma levels or AUC between the nasal sprays were recorded. However, we did observe a greater response after the 100-μL dose than after the 200-μL dose, indicating that dose concentration may be important. Plasma half-life ranged between 2.8 and 3.6 h and did not differ significantly between the methods. Time-to-peak plasma levels was ~50 min for all solutions. It is apparent, therefore, that the drug may continue to be absorbed for at least this period, which indicates the importance of retaining sufficient drug in the nasal cavity for this period of time.

Biological Response—The effects of the different methods of administration on circulating levels of F VIII:C and F vWF are shown in Figs. 4 and 5, respectively. Figure 4 shows the response in F VIII:C activity and, again, highlights the difference between the nasal sprays and drops. Peak response was 388 ± 170% and 269 ± 27% for the 100-μL and 200-μL nasal sprays, respectively. In contrast, the nasal drops administered by the rhinyle catheter and pipet resulted in maximum activity of 209 ± 118% and 201 ± 100%, respectively. This difference in maximum biological response be-

Table I—Pharmacokinetic Data after Nasal Administration of Desmopressin by Spray and Drops in Healthy Volunteers[a]

	AUC, μg/h	C_{max}, pg/mL	t_{max}, min	$t_{1/2}$, h
Spray (2 × 0.05 mL)	3675 ± 2098	675 ± 528	48 ± 11	3.1 ± 0.5
Spray (2 × 0.1 mL)	3556 ± 1848	567 ± 433	50 ± 8	3.6 ± 0.7
Rhinyle catheter (0.2 mL)	1599 ± 989	316 ± 223	55 ± 8	2.8 ± 0.7
Pipet (0.2 mL)	1318 ± 716	244 ± 161	50 ± 12	3.5 ± 0.2

[a] Desmopressin (300 μg); results are expressed as mean ± SD (n = 6).

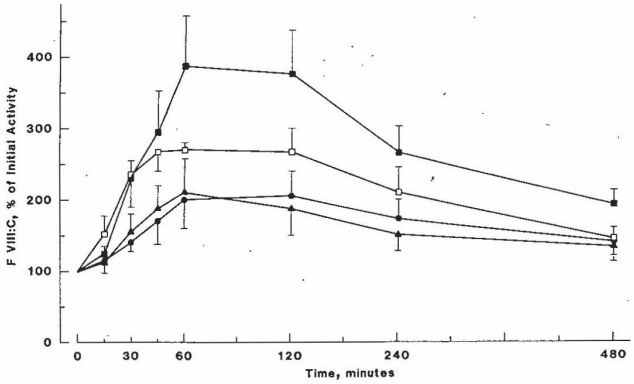

Figure 4—*The coagulant activity of the antihemophilia factor (F VIII:C) is expressed as a percentage rise of preadministration levels following nasal administration of 300 μg of desmopressin in 100 μL of spray (■—■), 200 μL of spray (□—□), and drops by a rhinyle catheter (●—●), and pipet (▲—▲) (mean ± SEM).*

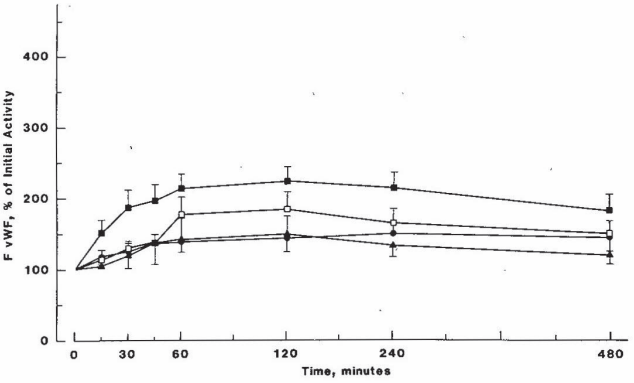

Figure 5—*The F VIII/von Willebrand factor (F vWF) is expressed as a percentage rise of preadministration levels following nasal administration of 300 μg of desmopressin in 100 μL of spray (■—■), 200 μL of spray (□—□), and drops by a rhinyle catheter (●—●), and pipet (▲—▲) (mean ± SEM).*

tween the nasal sprays and drops was highly significant ($p < 0.001$). Figure 5 shows a similar effect on F vWF activity, although the magnitude of response to all nasal administrations was less pronounced. This phenomenon is well known and has been described after intravenous administration of desmopressin.[14] The difference in peak activity between the nasal sprays and drops was again highly significant ($p < 0.001$). On plotting the individual maximum plasma levels against corresponding maximum rises in F VIII:C activity, a linear correlation was found ($r = 0.859$, $p < 0.001$), indicat-

ing that the biological response to desmopressin is indeed a function of its nasal absorption.

What is also evident is that the deposition and clearance data after nasal delivery by spray and drops can, for the first time, be interpreted in terms of their effect on drug absorption and biological response. These data suggest that a strong relationship exists between the rate of clearance and its absorption. Our findings indicate that the sprays, which were cleared at a slower rate than the drops, were better absorbed and, therefore, produced a more pronounced biological effect.

The increase in relative bioavailability seen after spray administration of desmopressin can be of considerable advantage to its therapeutic use. Desmopressin, the synthetic analogue of the naturally occurring antidiuretic hormone, vasopressin, has for many years been the drug of choice in the treatment of diabetes insipidus.[15] Traditionally, it has been delivered by the intranasal route using the rhinyle catheter method, and was one of the first examples of a peptide which could be given by the nasal route as a means of delivery to the systemic circulation. Recently, because of its ability to raise circulating levels of F VIII/vWF, it has an increasing place in the treatment of bleeding disorders such as hemophilia A and von Willebrand's disease.[14,16] It is in these indications that a greater magnitude of response has its most beneficial effect, as the levels of F VIII/vWF are strongly correlated with the bleeding tendency in these diseases. Previously, however, desmopressin has been administered by the parenteral route,[17] as earlier studies on the intranasal route gave unpredictable results with many poor responses. We have now established that the biological response is a function of nasal deposition, clearance, and absorption, and that absorption can be improved dramatically using sprays. Indeed, the levels attained here approach those described after intravenous administration of the recommended dose of 0.4 μg/kg body weight.[18]

The nasal sprays used in this study were of a different quality than those described elsewhere.[6] For example, when testing 10 samples of each spray pump, an average of five actuations were necessary to prime the pumps after which a series of accurate doses of 49 $\pm$ 3 μL and 101 $\pm$ 3 μL were achieved.

There are, of course, many factors which may affect absorption and biological response to peptides administered nasally. Unfortunately, many factors are outside our control, i.e., the state of the nasal function and accompanying pathologies such as allergic and chronic rhinitis. Indeed, it has been shown that even trivial conditions such as the common cold can alter clearance rates and thereby affect efficacy.[19] For this reason it is important to elucidate those factors which we can control, i.e., the method and technique of administration.

In conclusion, this study shows that an intranasal spray device can deposit well-controlled doses into the nostril and allow for delivery to the required site in the desired volume and concentration. Sprays are cleared at a slower rate than large drops from either intranasal pipets or rhinyle catheters, thereby producing a clear enhancement in absorption and bioavailability.

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Note Added in Proof

Since this paper was submitted, Stratford and Lee have published a paper (*Int. J. Pharm.* **1986**, *30*, 73–82) in which they demonstrate that the nasal cavity indeed may possess a potent metabolizing capacity especially for proteins and peptides. These results are in contrast to those reported previously (ref 1).