Effects of Viscous Hyaluronate–Sodium Solutions on the Nasal Absorption of Vasopressin and An Analogue

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The effects of viscous solutions of hyaluronate-sodium of various average molecular weights (MW) on the nasal absorption of vasopressin (AVP) and its analogue, 1-deamino-8-D-arginine vasopressin (1-d-8-DAVP), were examined in rats. Solutions of hyaluronate with MW greater than 3×10^5 daltons enhanced the nasal absorption of AVP; solutions of MW 5.5×10^4 daltons were not effective. The enhancing effects on the nasal absorption of AVP and 1-d-8-DAVP were dependent on the concentration in the range of 0–1.5% (w/v) hyaluronate (MW 1.4×10^6 daltons). The nasal absorption of AVP was increased with this solution at lower pH. Bioavailabilities after nasal administration of AVP and 1-d-8-DAVP in hyaluronate solutions (MW 1.4×10^6 and 2×10^6 daltons) increased more than 2- and 1.6-fold as compared to nasal administration of AVP and 1-d-8-DAVP in buffer solutions (pH 7.0), respectively. Hyaluronate solution (MW 1.4×10^6 daltons) did not affect the ciliary beat frequency of rabbit nasal mucosal membranes *in vitro*. Therefore, hyaluronate solution may be useful as a vehicle for nasal delivery of AVP and 1-d-8-DAVP.

KEY WORDS: hyaluronate-Na; nasal absorption; vasopressin; 1-deamino-8-D-arginine vasopressin.

INTRODUCTION

Oral administration of peptides is often limited by their instability and poor absorption in the gastrointestinal tract. The intranasal route may be useful for delivering peptides to the systemic circulation because of the large surface area of the nasal cavity, the highly vascularized bed of mucosa (1). The intranasal route is suitable for administration of a variety of drugs (up to a molecular weight of about 1000 daltons) without absorption enhancers. However, intranasally administered peptides of molecular weight greater than 1000 daltons show poor systemic availability (2). Important factors influencing intranasal absorption of peptides are the physicochemical nature of the nasal mucosa, formulation variabilities (pH, viscosity), and the peptides' resistance to tissue peptidases.

Intranasal administration of vasopressin (AVP; MW 1084 daltons) and its analogue, 1-deamino-8-D-arginine vasopressin (1-d-8-DAVP; MW 1069 daltons), has been widely used in the treatment of diabetes insipidus (3). In the present study, the effect of a viscous hyaluronate solution as absorption enhancer on the nasal administration of AVP or 1-d-8-DAVP was examined. Hyaluronate is a natural polymer and a major component of interstitial tissue. Solutions of hyaluronate are viscous and mucoadhesive (4).

MATERIALS AND METHODS

Materials. AVP and 1-d-8-DAVP were purchased from Sigma Chemical Co. (St. Louis, Mo.). Hyaluronates-Na (average MW 5.5×10^4, 3×10^5, 1.4×10^6, and 2×10^6 daltons) were obtained from Seikagaku Kogyo Co., Ltd. (Tokyo), Toyo Jozo Co., Ltd. (Shizuoka, Japan), Shiseido Co., Ltd. (Tokyo), and Kibun Food Chemical Co., Ltd. (Tokyo), respectively. All chemicals were reagent grade.

Preparations. Viscous hyaluronate solutions were prepared by presoaking hyaluronate (0.5, 1.0, and 1.5%, w/v) in isotonic 0.236 *M* citric acid:0.123 *M* disodium phosphate buffer (pH 4.0 and 5.0) and in isotonic 0.171 *M* potassium phosphate:0.144 *M* sodium acid carbonate buffer (pH 7.0). AVP and 1-d-8-DAVP were then dissolved in the viscous hyaluronate solution.

In a comparative study, AVP and 1-d-8-DAVP were dissolved in isotonic buffer (pH 7.0) or in 1% (w/v) sodium carboxymethyl cellulose (CMC) in isotonic buffer (pH 7.0) for nasal administration. They were dissolved in physiological saline (0.9%, w/v, NaCl) for intravenous administration.

The viscosity of preparations was measured by a cone-and-plate viscometer (E type, Tokyo Keiki Co., Ltd., Tokyo) at 37°C.

Animal Experiments. The nasal absorption of AVP or 1-d-8-DAVP was evaluated by its antidiuretic effect using the method described by Koyama *et al.* (5). Male Wistar rats (200–230 g) were fasted for 20 hr prior to the experiments. The rats were anesthetized by oral administration of ethanol to inhibit secretion of endogenous vasopressin. Three doses (separated by 30 min) of 10 ml/kg of 24% (w/v) ethanol were given orally through a gastric tube. The ethanol anesthetized rats were intubated to assist breathing, and then the left femoral vein was catheterized with polyethylene tubing. After a 10-ml/kg priming dose, a hypotonic solution containing 1.2% (w/v) ethanol, 1.7% glucose, and 0.3% (w/v) NaCl was administered through the femoral catheter as a constant infusion at 0.5 ml/kg/min. The urinary bladder was exposed by a small incision in the lower abdominal wall and polyethylene tubing was inserted into the bladder for urine collection.

Nasal administrations were made as described by Hirai *et al.* (6). The preparations (0.5 ml/kg body weight) were administered to the nasal cavity through a tube by a peristalic pump. The extent of antidiuresis was expressed as a percentage consisting of the ratio of urine volume produced after administration of the preparations to that produced in the 10-min period just before administration.

In a comparative study, AVP and 1-d-8-DAVP were administered intravenously at doses of 0.0025 IU/kg and 0.9 ng/kg, respectively.

Measurement of Nasal Ciliary Beat Frequency. The nasal mucociliary beat frequency was measured by a photo-

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Table I. Viscosity of Hyaluronate Solutions

Hyaluronate solution	MW of hyaluronate	Viscosity (cps)
1.0 (w/v%) pH 7.0	5.5×10^4	1.0
1.0 (w/v%) pH 7.0	3.0×10^5	20.3
0.5 (w/v%) pH 7.0	1.4×10^6	163.5
1.0 (w/v%) pH 7.0	1.4×10^6	1066.0
1.0 (w/v%) pH 7.0	2.0×10^6	1222.0
1.5 (w/v%) pH 7.0	1.4×10^6	2797.6

electric method described by Ohashi and Nakai (7). Nasal mucosal membranes from adult albino rabbits (2.0–2.5 kg) were used. Rabbits were sacrificed by air embolization, and the nasal mucosal membranes were removed and cut into square pieces (0.5 cm). The ciliary and beat frequency was measured in an intrinsic chamber filled with isotonic buffer or hyaluronate solution at 30 ± 0.5°C.

In Vitro *Evaluation of the Mucoadhesion of Hyaluronate Solutions*. The strength of mucoadhesion of hyaluronate solutions was measured by modification of a method described by Ch'ng *et al.* (8). Mucoadhesion was evaluated by measuring the force required to separate two nasal mucosal surfaces containing the preparation.

Data Analysis. The area above the percentage urine volume–time curve (AAC) was calculated by means of trapezoidal integration using the program MULTI (9). Bioavailabilities were calculated as $(AAC_{nasal}/AAC_{i.v.}) \times (D_{i.v.}/D_{nasal}) \times 100\%$, D being the dose of AVP or 1-d-8-DAVP. Statistical significance of antidiuretic effects of the preparations was assessed by using Student's paired t test.

RESULTS

Viscosities of Hyaluronate Solutions. The rheological characteristics of hyaluronate solutions measured with a cone-and-plate viscometer were such that the solutions may be classified as non-Newtonian liquids. The apparent viscosities of hyaluronate solutions (MW 1.4×10^6 daltons) increased with increasing hyaluronate concentration and with an increase in average molecular weight (1%, w/v, pH 7.0) (Table I).

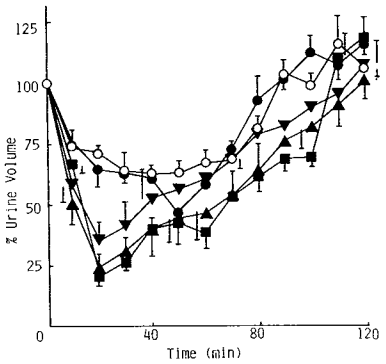

Fig. 2. Change in urine volume after nasal administration of AVP (0.025 IU/kg) in hyaluronate solution (pH 7.0) in rats. Average molecular weight (daltons) of hyaluronate: (●) 5.5×10^4; (▼) 3×10^5; (■) 1.4×10^6; (▲) 2×10^6. (○) Buffer solution (pH 7.0). Each point represents the mean ± SE of four animals.

Nasal Administration. The antidiuretic effects of nasal administration of AVP (0.025 IU/kg) in buffer solution (pH 7.0), CMC solution (1%, w/v, pH 7.0), and hyaluronate (MW 1.4×10^6 daltons) solution (1%, w/v, pH 7.0) in rats are shown in Fig. 1. The antidiuretic effect after nasal administration of AVP with hyaluronate solution was greater than with buffer solution (pH 7.0) or CMC solution. The peak of antidiuretic effects with the hyaluronate solution was obtained 20 min after nasal administration, compared to 40 min with the other preparations.

The effects of the molecular weight of hyaluronate (1%, w/v, pH 7.0) on the antidiuretic effects of AVP (0.025 IU/kg) in rats after nasal administration are shown in Fig. 2. The antidiuretic effects were in the following rank order of hyaluronate molecular weights (daltons): $2 \times 10^6 = 1.4 \times 10^6 > 3 \times 10^5 > 5.5 \times 10^4$. The maximum antidiuretic effects occurred 20 min after nasal administration (except for the MW 5.5×10^4 dalton solution).

The influence of the concentration of hyaluronate (MW 1.4×10^6 daltons) on the antidiuretic effects of AVP (0.025 IU/kg) and 1-d-8-DAVP (9 ng/kg) in rats after nasal administration is shown in Figs. 3 and 4, respectively. Greater antidiuretic effects were observed with higher concentra-

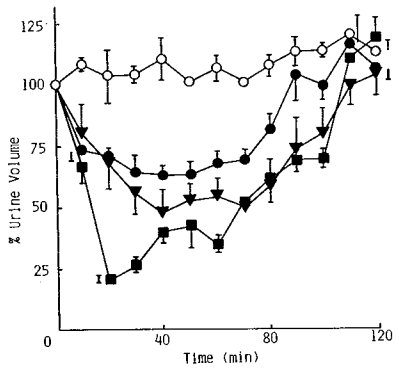

Fig. 1. Change in urine volume after nasal administration of AVP (0.025 IU/kg) in various vehicles (pH 7.0) in rats. (○) Buffer solution alone; AVP in (●) buffer solution, (■) 1.0% (w/v) hyaluronate (average MW 1.4×10^6 daltons) solution, and (▼) 1.0% (w/v) CMC solution. Each point represents the mean ± SE of four animals.

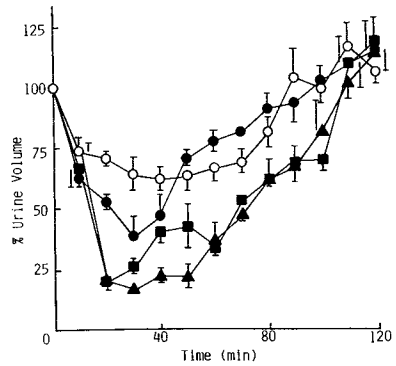

Fig. 3. Change in urine volume after nasal administration of AVP (0.025 IU/kg) in hyaluronate solutions (average MW 1.4×10^6 daltons, pH 7.0) of various concentrations in rats. Concentrations of hyaluronate: (○) 0%, w/v; (●) 0.5%, w/v; (■) 1.0%, w/v; (▲) 1.5%, w/v. Each point represents the mean ± SE of four animals.

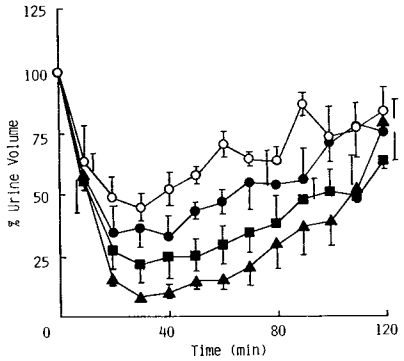

Fig. 4. Change in urine volume after nasal administration of 1-d-8-DAVP (9 ng/kg) in hyaluronate solutions (average MW 1.4×10^6 daltons, pH 7.0) of various concentrations in rats. Concentrations of hyaluronate: (○) 0% w/v; (●) 0.5%, w/v; (■) 1.0%, w/v; (▲) 1.5%, w/v. Each point represents the mean ± SE of four animals.

tions of hyaluronate (0–1.5%, w/v). The antidiuretic effects after nasal administration of 1-d-8-DAVP were prolonged compared with those of AVP.

The effects of the pH of hyaluronate (MW 1.4×10^6 daltons) solutions (1%, w/v) on the antidiuretic effects of AVP (0.025 IU/kg) in rats after nasal administration are shown in Fig. 5. The antidiuretic effects of AVP and 1-d-8-DAVP after nasal administration in hyaluronate solutions were greater than after administration in buffer solutions at various pH. Greater antidiuretic effects were shown with lower pH of hyaluronate or buffer solutions of AVP. However, the antidiuretic effects of 1-d-8-DAVP were not influenced by the pH of the hyaluronate solutions (data not shown).

The bioavailabilities in rats after nasal administration of AVP and 1-d-8-DAVP in hyaluronate solutions are summarized in Table II. Bioavailabilities of AVP and 1-d-8-DAVP in hyaluronate solutions were increased more than 2- and 1.6-fold as compared with buffer solutions (pH 7.0), respectively.

Nasal Mucociliary Beat. The frequency of the ciliary beat in rabbit nasal mucosal membranes *in vitro* was not affected by hyaluronate solutions (MW 1.4×10^6 daltons, 0.5%, w/v) at various pH's (data not shown).

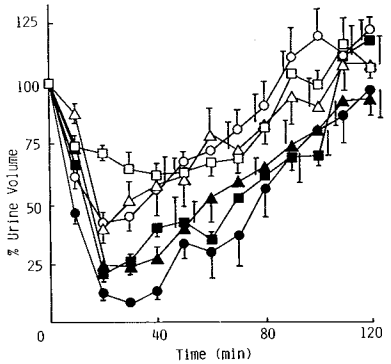

Fig. 5. Change in urine volume after nasal administration of AVP (0.025 IU/kg) in hyaluronate (average MW 1.4×10^6 daltons) (1.0%, w/v) or buffer solutions at various pH in rats. Buffer solution pH: (○) pH 4.0; (△) pH 5.0; (□) pH 7.0. Hyaluronate solution pH: (●) pH 4.0; (▲) pH 5.0; (■) pH 7.0. Each point represents the mean ± SE of four animals.

Table II. Bioavailabilities (BA) After Nasal Administration of AVP and 1-d-8-DAVP in Hyaluronate Solutions in Rats[a]

	AAC (% urine volume · min)	BA (%)
AVP (0.025 IU/kg)		
Buffer solution (pH 7.0)	2506.3 ± 308.5	6.8 ± 0.8
Hyaluronate solution		
1.0 (w/v%), pH 7.0		
(1.4×10^6)	4704.6 ± 61.5	12.7 ± 0.2**
1.5 (w/v%), pH 7.0		
(1.4×10^6)	5099.0 ± 81.1	13.7 ± 0.2**
1.0 (w/v%), pH 4.0		
(1.4×10^6)	5405.2 ± 639.6	14.6 ± 1.7*
1.0 (w/v%), pH 7.0		
(3.0×10^5)	3693.1 ± 341.5	10.0 ± 1.0*
CMC solution		
1.0 w/v%, pH 7.0	3445.4 ± 457.5	9.3 ± 1.2
1-d-8-DAVP (9 ng/kg)		
Buffer solution (pH 7.0)	3363.7 ± 529.5	6.0 ± 0.9
Hyaluronate solution		
1.0 (w/v%), pH 7.0		
(1.4×10^6)	5655.5 ± 714.9	10.1 ± 1.3*
1.5 (w/v%), pH 7.0		
(1.4×10^6)	6530.0 ± 412.3	11.4 ± 0.9*

[a] The AAC was calculated using the trapezoidal method. BA was calculated as $(AAC_{nasal}/ACC_{i.v.}) \times (D_{i.v.}/D_{nasal}) \times 100\%$, D being the dose of AVP or 1-d-8-DAVP. Numbers in parentheses indicate the average molecular weight (daltons) of hyaluronate. Each value represents the mean ± SE of four animals.
* Significantly different from buffer solution (pH 7.0) at $P < 0.05$.
** Significantly different from buffer solution (pH 7.0) at $P < 0.001$.

Mucoadhesion of Hyaluronate Solutions. Mucoadhesive properties of hyaluronate solutions to rabbits nasal mucosa are shown in Table III. The mucoadhesive strength of hyaluronate (MW 1.4×10^6 daltons) solutions increased with an increase in hyaluronate concentration. Furthermore, the mucoadhesive strength of hyaluronate solutions (1%, w/v,

Table III. Evaluation of the Mucoadhesive Properties of Hyaluronate Solutions[a]

	MW of hyaluronate	Detachment force/area (dyne/cm^2)
Hyaluronate solution		
0.5 (w/v%), pH 7.0	1.4×10^6	9103.0 ± 1154.5
1.0 (w/v%), pH 7.0	1.4×10^6	11530.4 ± 173.4
1.5 (w/v%), pH 7.0	1.4×10^6	14275.8 ± 621.8*
1.0 (w/v%), pH 7.0	5.5×10^4	6764.6 ± 260.2**
1.0 (w/v%), pH 7.0	3.0×10^5	10576.8 ± 500.5
1.0 (w/v%), pH 7.0	2.0×10^6	12859.8 ± 202.3
CMC solution		
1.0 (w/v%), pH 7.0		5750.8 ± 256.8**

[a] Each value represents the mean ± SE of three experiments.
* Significantly different from hyaluronate solution (pH 7.0) at $P < 0.05$.
** Significantly different from hyaluronate solution (pH 7.0) at $P < 0.001$.

pH 7.0) increased with an increase in the average molecular weight of hyaluronate. This effect was greater for hyaluronate solutions than for the CMC solution (1%, w/v, pH 7.0).

DISCUSSION

Generally, polypeptides are poorly absorbed across mucosal membranes. In this study, AVP and 1-d-8-DAVP in buffer solutions (pH 4.0–7.0) were slightly absorbed from the nasal cavity. Hyaluronate (MW 3×10^5, 1.4×10^6, and 2×10^6 daltons) solutions enhanced the nasal absorption of AVP and 1-d-8-DAVP. The enhancing effects on the nasal absorption of AVP and 1-d-8-DAVP were dependent on the hyaluronate concentration in the range of 1–1.5% (w/v) (MW 1.4×10^6 daltons). Solutions of hyaluronate of lower molecular weight (MW 5.5×10^4 daltons) did not enhance the nasal absorption of AVP. These absorption enhancing effects of hyaluronate solutions were lower than those seen with other enhancers, such as bile salts or laureth-9 (10).

The nasal absorption of AVP was greater with lower pH of the hyaluronate (MW 1.4×10^6 daltons) and buffer solutions (pH 4.0–7.0). This result is consistent with the nasal absorption of secretin (MW 3052) (11). The effect of pH on the nasal absorption of secretin was due mainly to changes in the structure or electrical charge of the membrane and/or to self-association and conformational changes in secretin. The pH dependency of nasal AVP absorption may be due to one of these mechanisms. However, the nasal absorption of 1-d-8-DAVP was not affected by pH. The reasons for this discrepancy are unclear.

Solutions of hyaluronate with MW greater 3×10^5 daltons had a high viscosity of mucoadhesion. We reported previously that an aqueous gel of polyacrylic acid (Carbopol or Hiviswako) significantly enhanced the absorption of insulin and a calcitonin analogue when administered rectally (12), vaginally (13), and nasally (14). Although the rheological properties and mucoadhesion of polyacrylic acid gel and hyaluronate solution are similar, the mechanism of absorption enhancement by these polymers is still unclear.

For nasal drug delivery, the nasal cilliotoxicity of the drug and the formulation are important (15,16). Since the mucociliary clearance of the nose removes dust, allergens, and bacteria, it should not be influenced by nasal medication. Hermens *et al.* reported that absorption enhancers such as dehydroxy bile salt and laureth-9 caused very rapid and irreversible ciliostatic effects on human adenoid tissue *in vitro* (16). In the present study, hyaluronate solutions (0.5%, w/v, pH 4.0–7.0) did not affect the nasal mucociliary beat in rabbits *in vitro*.

In conclusion, viscous hyaluronate solutions showed a moderate enhancing effect on nasal absorption of AVP and 1-d-8-DAVP. Therefore, this solution may be useful as a vehicle for nasal delivery of polypeptides.

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