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Transdermal iontophoretic delivery of arginine-vasopressin (I): Physicochemical considerations

Parichat Lelawongs, Jue-Chen Liu, Ovais Siddiqui and Yie W. Chien

Controlled Drug-Delivery Research Center, College of Pharmacy, Rutgers University, Piscataway, NJ 08855-0789 (U.S.A.)

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Summary

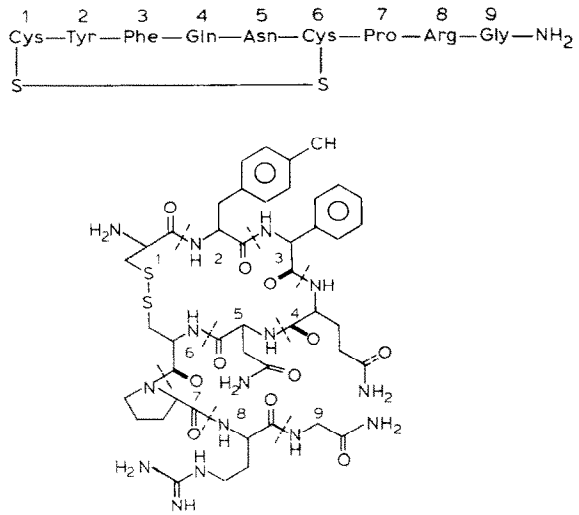
The enhancement in the transdermal delivery of arginine-vasopressin (AVP), a nonapeptide antidiuretic hormone (ADH), was studied using the Transdermal Periodic Iontotherapeutic System (TPIS). The TPIS is capable of generating a pulse current at a specific combination of waveform, frequency and ON/OFF ratio. The in vitro permeation studies of AVP across the hairless rat skin was conducted under TPIS application and the results were compared with that by passive diffusion. Vasopressin was found to be relatively stable during the course of the experiment. The effect of pH and ionic strength of the donor solution and drug concentration on the AVP flux was evaluated. Under the application of TPIS, the transdermal delivery rate of AVP could be enhanced as much as 1000-fold and the degree of enhancement was noted to be dependent upon the physicochemical conditions used during the iontophoresis treatment. The flux of AVP at pH 5.0 and 7.4 was similar at constant ionic strength, but increased substantially as the ionic strength decreased. A linear relationship was established between AVP flux and AVP concentration in the donor compartment. The skin permeability enhanced by TPIS treatment is reversible and upon termination of iontophoretic application, the AVP flux was observed to return to that by passive diffusion alone.

Introduction

The use of iontophoresis technique to facilitate the systemic delivery of charged drugs across the biological membranes has recently been the subject of a number of studies (Bellantone et al., 1986; Liu et al., 1988; Sanderson et al., 1987; Siddigui et al., 1985; Tyle, 1986). These studies have shown that iontophoresis can be used to enhance the transdermal delivery of uncharged or charged molecules, including macromolecules. Few studies, however, have assessed the importance of pH and ionic strength of the vehicle and drug concentration in donor solution on the transdermal permeation of peptide/protein drugs.

Arginine-vasopressin (AVP), a nonapeptide hormone, has an amino acid sequence and chemical structure as that shown in Fig. 1. The structure of AVP can be divided into two parts: a hexapeptide ring, in which 2 cysteine residues form a disulfide bridge, and a tripeptide tail. Owing to the presence of the basic arginine and the blocking of C-terminus with $-NH_2$, AVP has an isoelectric point as high as 10.9 (Butt, 1975). It has a molecular weight of 1084; with a calculated diameter of 1.6 nm in aqueous solution (Walter et al., 1974), which is small as compared to 20–100

Correspondence: Y.W. Chien, Controlled Drug-Delivery Research Center, College of Pharmacy, Rutgers University, Piscataway, NJ 08855-0789, U.S.A.



- MW = 1084
- Isoelectric point = 10.9
- Approximate diameter in aqueous solution is 16 A°, comparable with 20-100 μ of those of sweat glands and hair follicles

Fig. 1. Chemical structure of arginine vasopressin.

μm for sweat glands and hair follicles (Bronaugh et al., 1982; Stutten et al., 1981).

The objective of this study was to examine the effect of physicochemical parameters, like pH, ionic strength and drug concentration on the iontophoretic transport of AVP through the excised hairless rat skin.

Materials and Methods

Materials

All chemicals were used as received. Synthetic arginine-vasopressin (AVP), as an acetate salt (each mole of AVP contains 2 moles of acetate and 2 moles of water, mol. wt. = 1238) (Sigma Chemical Co., St. Louis, MO), was used. Radiolabelled AVP, [phenylalanyl-3,4,5-^{3}H(N)]AVP, with a sp. act. of 70.0 Ci/mmol was purchased from New England Nuclear (Boston, MA) and was used as a tracer during the in vitro skin permeation studies. The radiolabelled AVP had a purity of approximately 99% and was used within 6–8 weeks from the date of its manufacture. Biofluor (New England Nuclear, Boston, MA) was used as a liquid scintillation cocktail. A liquid scintillation counter (LKB 1214, Gaithersburg, MD) was used for radioactivity counting.

Platinum wires (99.95% purity, 1 cm × 0.5 mm, Johnson Mattey, Seabrook, NH) were chosen as the electrodes in our permeation experiments; even though the platinum electrodes had been known to cause pH drift and gas bubbling in the solution when high current densities were applied. The preliminary studies have shown that they do not precipitate peptides/proteins like Ag/AgCl electrodes do.

Stability studies of AVP in solution

The stability of AVP in both citrate–phosphate buffer at pH 5.0 and 7.4 and isotonic Sorensen phosphate buffer at pH 7.4 was conducted, using 4 drug concentrations (100, 50, 25 and 12.5 μg/ml) at 37°. The solution of AVP was placed in the test tubes, tightly capped, and then immersed in a water bath. The samples were taken at regular intervals and stored at 0–4° C prior to analysis by HPLC. All the glassware used in this study was silanized with Sylon CT (Supelco, Bellefonte, PA) to prevent the adsorption of AVP to the glass surface.

The stability of AVP in the donor vehicles under iontophoresis treatment was also investigated. The study was conducted in the V-C diffusion cell with the donor compartment containing freshly-prepared AVP solution at a specific pH. Either an excised rat skin or an aluminum foil was used as a membrane placed between the donor and receptor compartments. The current was applied through a pair of platinum electrodes. A similar setup without iontophoresis treatment was also run as a control. The samples were taken at regular intervals and analyzed by HPLC.

The HPLC assay procedure modified from the method reported earlier (Rao, 1986) was used. The assay was carried out using HP 1090 Liquid Chromatographic system (Hewlett Packard) equipped with a polymeric column: reversed-phase styrene/DVB (PLRP-S, 10.0 nm column, 5 μm, 250 × 4.6 mm; Polymer Laboratories, Amherst, MA), a diode-array detector operating at 215 nm, and a HP 3392A integrator. The volume of injection was 10 μl. A mixture of acetonitrile and 0.05

M ammonium acetate buffer (pH 6.5) at a ratio of 25 : 75 was used as the mobile phase. At a flow rate of 1 ml/min, a peak was observed for AVP at 3.5 min. Quantitation was accomplished based on the calibration curve which was found to be linear from 2.5 to 100 μg/ml.

In vitro iontophoretic skin permeation

The freshly-excised skin, obtained from the abdominal region of hairless rats (HRS strain; Armed Forces Institute of Pathology, Washington, DC), was mounted between the donor and receptor compartments of V-C skin permeation cells (Crown Glass, Somerville, NJ). The area of the skin available for drug permeation was 0.64 cm^2. The solution was stirred by a match pair of starhead-shaped magnets rotating at 600 rpm and the temperature was maintained at 37°C. The donor and receptor compartments were filled with the solution of 3.0 ml each. The donor compartment contained freshly prepared buffer solution of cold AVP and radiolabelled AVP, at a specific pH.

The AVP permeation studies were performed using a citrate–phosphate buffer (McIlvaine), either at pH 5 or 7.4. The pH 5 buffer consisted of 0.103 M Na_2HPO_4 and 0.048 M citric acid. The pH 7.4 buffer consisted of 0.182 M Na_2HPO_4 and 0.009 M citric acid. The ionic strength of these buffers was 0.25 and 0.50 M, respectively (Elving et al., 1956). The isotonic Sorensen phosphate buffer of 0.013 M NaH_2PO_4, 0.053 M Na_2HPO_4 and 0.075 M NaCl at pH 7.4 was used as the receptor solution.

A pair of platinium electrodes were immersed in the solutions with the anode in the donor compartment and the cathode in the receptor compartment. The distance between electrodes and skin was consistent. These electrodes were connected to an adjustable constant-current power source, called the Transdermal Periodic Iontotherapeutic System, which has 6-channels and is capable of delivering a synchronized output of pulse current for two sets of triplicate experiments. The current profiles of pulse DC can be controlled at a specific combination of waveform, frequency, ON/OFF ratio, intensity and duration. In this investigation, the square-waveform pulse DC with a frequency of 2 kHz and ON/OFF ratio of 1/1 was used. The applied current was at either periodic or continuous manner. The current applied in periodic manner was ON for 10 min and then OFF for 30 min as a cycle for 6 cycles. The continuous-mode current application was also carried out with the current applied continuously for 4 h.

Samples (0.5 ml each) were taken at appropriate intervals from the receptor solution and immediately replaced with 0.5 ml of fresh buffer solution right after each sampling. The samples were mixed with scintillation cocktail and their radioactivity were counted in the liquid scintillation counter. The corrected dpm values were then used to calculate the amount of AVP permeating through the skin specimen. The amount of drug permeating through the skin was monitored during the current application and then for another 5 h after the termination of current treatment.

The purity and identity of radiolabelled AVP in the donor and receptor solutions were evaluated by thin-layer chromatography (TLC). In brief, the solution was spotted on a silica gel plate, previously cut into strips. The mixture of *n*-butanol/pyridine/acetic acid/water (30 : 20 : 6 : 24) was used as the mobile phase. Following completion of the TLC process, the plate was dried at room temperature and the strip was cut into equal divisions, approximately 1.5 cm long each. The silica gel was scraped off the plate and collected into the vials containing scintillation cocktail and radioactive counts were measured.

Effect of pH. The effect of pH on the skin permeation of AVP was conducted at pH 5 and 7.4, with and without adjusting the ionic strength of the donor solution. Pulse DC with a current density of 0.78 mA/cm^2 was applied in a periodic manner.

Effect of ionic strength. The effect of ionic strength on the skin permeation of AVP was studied using citrate–phosphate buffer at pH 7.4, with the ionic strength in the donor solution varying from 0.06 to 0.50 M. Pulse DC with a current density of 0.78 mA/cm^2 was applied in a periodic manner.

Effect of concentration. The experiment was conducted at various concentrations of AVP

acetate, ranging from 12.5 μg/ml (0.01 M) to 100 μg/ml (0.08 M), at pH 5.0. The effect of AVP concentration was conducted at two levels of ionic strength, approximately 0.25 and 0.06 M, in the donor solution. Pulse DC with a current density of 0.47 mA/cm^2 was applied continuously for 4 h.

Solution conductivity measurement

The solution of AVP acetate in the deionized water was prepared at different concentrations ranging from ~ 0.4 to 4.0 mM. One ml of solution was put in a test tube thermostatted at 37 °C in a water bath. The conductivity of AVP in the solution was measured by a conductivity meter (CDM 83, Radiometer, Copenhagen, Denmark). Prior to the measurement, the conductivity cell (Microelectrodes, Londonderry, NH) was calibrated using a reference solution which had a conductivity in the 0.10–1.3 mS/cm range. Triple determinations of the conductivity were made for each solution.

The citrate–phosphate buffers (pH 5.0 and 7.4) were prepared at different ionic strengths and their conductivity was then determined by the same procedure. The cell was calibrated using the reference solutions with the conductivity of 10–130 mS/cm.

Results and Discussions

Stability of AVP solution

Fig. 2 shows the stability profiles of AVP in different buffer solutions at 37 °C. The results indicate that the degradation of AVP in a citrate–phosphate buffer at pH 5.0 and 7.4 and in an isotonic Sorensen phosphate buffer at pH 7.4 follows first-order kinetics with the mean degradation constant of 0.0007, 0.0015 and 0.0035 h^{-1}, respectively. It suggests that 0.63%, 1.34%, and 3.10% AVP is degraded over the course of permeation study.

The AVP in solutions was also found to be stable under the iontophoresis treatment. When an aluminium foil was used as the partition, less than 10% of the drug was found to be degraded at a current intensity of 0.3 mA for a 4-h period. However, the disappearance rate of AVP in solution was greater when the skin was used. This

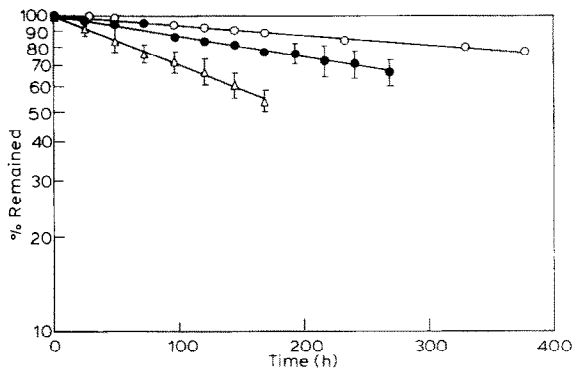


Fig. 2. Stability profiles of arginine-vasopressin in buffer solutions at 37 °C. Key: (○) citrate-phosphate buffer, pH 5.0; (●) citrate-phosphate buffer, pH 7.4; (△) Sorensen phosphate buffer, pH 7.4.

increased loss of AVP in solution was found later to be a result of the migration and accumulation of drug in the skin during iontophoresis treatment (Lelawongs et al., 1989). In addition, the adsorption of AVP on the surface of the diffusion cell was found to be negligible during the course of permeation study.

In vitro iontophoretic permeation study

Effect of pH. According to Nernst-Planck equation, the flux of charged species under the electrical field is governed by both the chemical and electrical potential gradients (Lakshiminarayanaiah, 1984). Since AVP has a relatively high isoelectric point (pI = 10.9), more than 99% of the drug is protonated in a buffer solution with pH lower than 9.0. Under this condition, the electrical potential gradient across the skin is apparently the main driving force. As illustrated in Fig. 3, no significant difference in AVP flux was observed at pH 5.0 and 7.4 when an equal ionic strength (~ 0.25 M) was maintained in the buffer solutions. On the other hand, when the ionic strength was not maintained at the same level, a greater AVP flux was observed at pH 5.0 than at pH 7.4. This is due to the effect of variation in the ionic strength, not due to the difference in solution pH of the system. The effect of ionic strength will be addressed later.

The pH of drug solution affects not only the ratio of charged and uncharged drug species, but

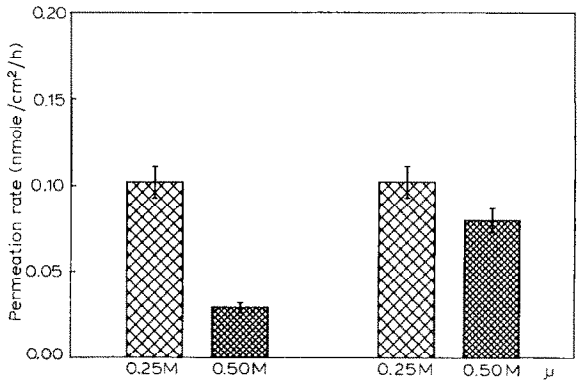


Fig. 3. Effect of pH of donor solution on permeation rate of arginine-vasopressin at steady-state. Key: (⊠) pH = 5.0; (▩) pH 7.4. Pulse currrent of 0.78 mA·cm^{-2} was applied in a periodic manner for 6 cycles.

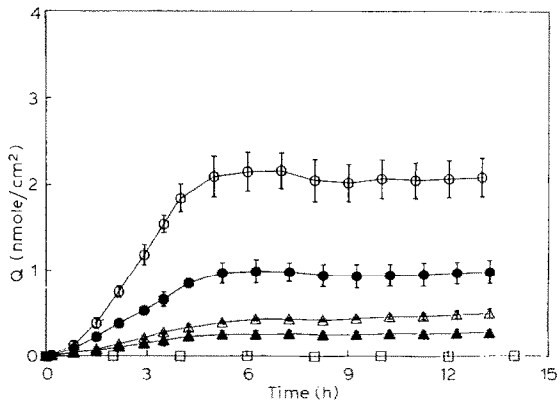


Fig. 4. Skin permeation profile of arginine vasopressin at different ionic strengths. Key: (○) 0.06 M; (●) 0.12 M; (△) 0.25 M; (▲) 0.50; (□) passive diffusion. The donor solution had a pH of 7.4. A pulse current of 0.78 mA·cm^{-2} was applied in a periodic manner for 6 cycles.

also the property of the skin surface. It is known that the skin consists of a heterogeneous mixture of proteins which can be charged in either direction because of their amphoteric characters (Rothman, 1954). The skin surface has a minimum charge density at about pH 3–4, which is about the isoelectric point of keratin in the stratum corneum layer (Schade and Marchionini, 1927). At pH greater than 4, skin carries a negative charge and acts as a cation-selective membrane (Burnette and Marrero, 1986; Rosendal, 1943), favoring the permeation of protonated AVP. However, as pH is lower than 3, the skin becomes a positively-charged membrane and prefers the transport of anionic species. This factor should be taken into consideration when one formulates the vehicle for iontophoretic delivery system.

Effect of ionic strength. It is expected that the variation of ionic strength in the donor solution should be of importance for ionotophoretic transport. The protonated AVP makes only a small contribution to the total ionic strength of the donor solution, so its effect can be ignored. The studies on the effect of the ionic strength on AVP skin permeation was conducted by varying the concentration of buffering species in the donor solution. The results in Fig. 4 show that the iontophoresis-facilitated skin permeation profile of AVP decreases as the ionic strength of the donor solution increases. The skin permeation fluxes and enhancement factors are both declined as the ionic strength of the donor solution increases (Table 1). In other words, the skin permeation rate of AVP was enhanced by iontophoresis treatment by 50-fold at the ionic strength of 0.50 M. The magnitude of enhancement was further increased to approximately 500-fold when the ionic strength was reduced by about 8 times to 0.06 M with the current density at 0.78 mA/cm^2.

One of the explanations for this phenomenon is the competition of drug ions and buffer ions for the current applied. Since most of the current

TABLE 1

Effect of ionic strength of donor solution on the iontophoretic permeation rate of arginine-vasopressin

Ionic strength (M)	Flux [a] (nmol/cm^2/h)	Enhancement factor [b]
0.06	0.585 (±0.060)	487.5 (±50.0)
0.12	0.244 (±0.020)	203.3 (±16.7)
0.25	0.090 (±0.007)	75.0 (±5.8)
0.50	0.060 (±0.010)	50.0 (±8.3)

[a] Mean (±S.D.).

[b] Enhancement factor = $\frac{\text{Skin permeation flux}_{(\text{Iontophoresis})}}{\text{Skin permeation flux}_{(\text{Passive})}}$

where passive permeation rate = 0.0012 nmol/cm^2/h.

would be carried by buffer ions with relatively high mobilities, the actual fraction of the applied current carried by AVP ions would be proportionally reduced as the concentration of buffering agent in the donor solution increases, resulting in lower skin permeation rate.

In addition to the competition between AVP and buffering ions for the current, the activity coefficient of the drug can also be changed with the variation of ionic strength in the donor solution. By using the extended Debye-Hückel equation and knowing that the net charge of AVP equals 2, the activity coefficient of AVP ion in the aqueous solution can be estimated. For example, when the donor solution has an ionic strength of 0.50 M, the protonated AVP has a calculated activity coefficient of 0.37. The activity coefficient was found to increase by 65% when the ionic strength was reduced from 0.50 to 0.06 M. The increase in the activity coefficient of protonated AVP in response to the reduction in the concentration of competitive ions could cause an increase in the skin permeation of the drug. After reanalyzing the data by using the extended Debye-Hückel equation, a linear relationship can be established between the permeation rate of AVP and its activity (as a function of ionic strength) within a range of ionic strengths (Fig. 5). As expected, a deviation from the linearity is observed at a very high ionic strength.

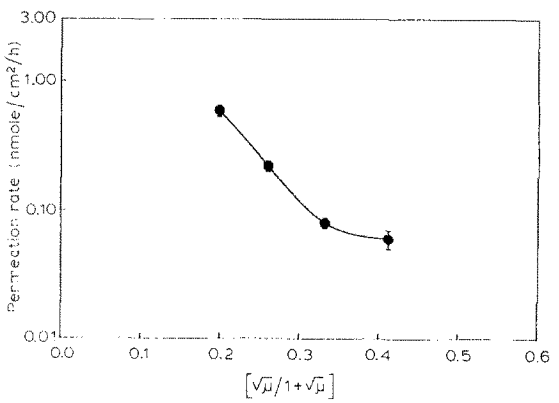


Fig. 5. Relationship of the steady-state permeation rate of arginine-vasopressin and its activity coefficient, γ ($\log \gamma \cdot \alpha - \sqrt{u}/1+\sqrt{u}$).

TABLE 2

Conductivity of AVP acetate in aqueous solution at various concentrations

AVP concentration (mM)	Conductivity [a] (μS/cm)	Molar conductance [b] ($cm^2 \cdot S/mol$)
4.04	408.16	101.06
3.03	319.66	105.53
2.02	218.10	108.00
0.81	90.21	111.68
0.40	44.58	110.38

[a] At 37 °C.

[b] Equivalent conductance of acetate at infinite dilution = 48 $cm^2 \cdot S/mol$.

For AVP:
(1) Equivalent conductance at infinite dilution = 7 $cm^2 \cdot S/mol$.
(2) Ionic mobility = 7.2×10^{-5} $cm^2 \cdot S/Coulomb$.
(3) Diffusivity = 9.7×10^{-7} cm^2/s.
Unit: 1 $cm^2 \cdot S/Coulomb = 1$ $cm^2/s/V$.

It is also possible that AVP might form an ion-pair with buffer species in the solution, resulting in a higher passive flux. In our study, we did not explore this phenomenon in detail. However, in the presence of iontophoresis, the AVP flux might decrease as the concentration of anions, such as phosphate or citrate, increases since ion-pair formation between AVP and these buffer species would lower the percentage of AVP in the free-ionized form. Depending on the degree of the association, the energy involved during current application might perturb the formation of ion pairs. The lower the attracting force, the less the contribution of ion-pairing on the decrease in iontophoretic flux of AVP and vice versa.

In order to ensure that the effect of ionic strength on the skin permeation rate of AVP is not complicated by the effect of pH drift in the system during current application, the pH was measured at both the beginning and the end of the experiment. The pH value was found to remain relatively unchanged for all the ionic strength studies.

The conductivity of solutions was measured and the results supported the explanation of current competition. Table 2 provides the molar conductivity of AVP in solution at different concentrations. Since AVP used in the study is an

TABLE 3

Calculated conductivity of protonated AVP at extremely low concentration range

AVP concentration (mM)	Conductivity [a] (μS/cm)
0.08	1.13
0.04	0.56
0.02	0.28
0.01	0.14

[a] At 37 °C.

acetate salt form, the conductivity measured is the sum of the conductivities contributed by the protonated AVP and the acetate ions. At extremely low concentrations, the interaction of these ions in solution is expected to be negligible, hence the molar conductivity of AVP acetate is relatively constant (Table 2). The molar conductivity (or equivalent conductivity) of AVP acetate at infinite dilution is approximately 110 $cm^2 \cdot S/mol$. Based on the conductivity values reported in the literature, the equivalent conductivity of acetate at infinite dilution at 37 °C is calculated to be ~ 48 $cm^2 \cdot S/mol$ (Weast et al., 1985-86). In the AVP molecule, the pK_a of $-NH_2$ group in cysteine (position 1) and =NH is arginine (position 8) are 9.0 and 12.9, respectively. Consequently, almost 100% of the drug existed as AVP^{2+} at the experimental pHs used in this investigation. The equivalent conductivity, Λ, of AVP was then calculated to be 7 $cm^2 \cdot S/mol$ which is about 7 times lower than that of the competing ion, sodium ion, in the donor solution. This analysis enables us to calculate the conductivity of protonated AVP in the solution at lower concentrations, at which the measurement cannot be made precisely. The calculated conductivity of AVP in the solution is given in Table 3. In addition, the ionic mobility, u, and diffusivity, D, of protonated AVP in the solution can be obtained by using the following relationships:

$$u_{AVP} = \frac{\Lambda_{AVP}}{F} \tag{1}$$

$$D_{AVP} = \frac{u_{AVP} \cdot R \cdot T}{z \cdot F} \tag{2}$$

where $R = 8.314$ $J \cdot mol^{-1} \cdot K^{-1}$ (1 J = 1 volt · coulomb); T = absolute temperature (310 K); z = charge of protonated AVP (+2); F = Faraday constant (96,500 Coulomb · mol^{-1}).

Using Eqns. 1 and 2, the mobility and diffusivity at 37 °C are calculated to be 7.2×10^{-5} $cm^2 \cdot S/Coulomb$ ($cm^2/s/V$) and 9.7×10^{-7} cm^2/s, respectively.

The skin permeation flux of AVP is directly proportional to the fraction of current carried by AVP ions in both the solution and the skin, which is known as the transport or transference number, t_{AVP}.

$$t_{AVP} = \frac{I_{AVP}}{(\Sigma I)} \tag{3}$$

As $I_{AVP} = C_{AVP} \cdot F \cdot z_{AVP} \cdot u_{AVP}$ (equal to conductivity) dV/dx, t_{AVP} in terms of ion parameters can be rewritten as:

$$t_{AVP} = \frac{C_{AVP} \cdot z_{AVP} \cdot u_{AVP}}{C_{AVP} \cdot z_{AVP} \cdot u_{AVP} + \Sigma C_i \cdot z_i \cdot u_i + \Sigma C_j \cdot z_j \cdot u_j} \tag{4}$$

where t_{AVP} = transference number of AVP in solution; C = concentration in solution or skin; i = competitive ions present in the donor solution; j = competitive ions present in the receptor solution.

Eqn. 4 indicates that the transference number of AVP is related to its mobility and to the mobility of other competitive ions. The moving velocity of the ions depends on their size and charge density as well as the degree of hydration. The faster the competitive ions move, the less the fraction of current is carried by AVP, resulting in lower AVP flux. In addition, the concentration of competitive ions in both donor and receptor solutions also affect the transference number of AVP. Therefore, for iontophoretic transport studies, it is necessary to state the experimental conditions.

For simplicity, we consider only the fraction of anodal current carried by the drug ion in the donor solution since the skin acts as a separator

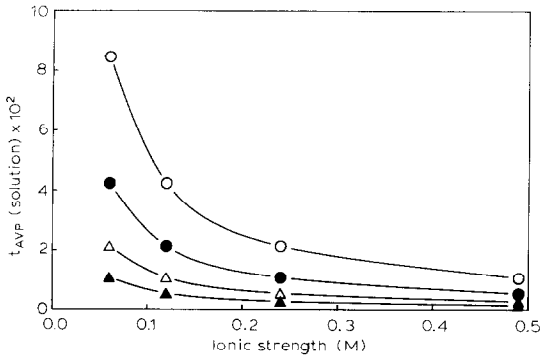


Fig. 6. Effect of ionic strength of donor solution on the solution transference number of arginine-vasopressin (t_{AVP}) at different AVP concentrations. Key: (○) 0.08 mM; (●) 0.04 mM; (△) 0.02 mM; (▲) 0.01 mM.

TABLE 4

Calculated t_{anodal} of AVP in donor solution and skin at different ionic strengths

Ionic strength (M)	t_{anodal} [a] (solution) ($\times 10^2$)	t_{AVP} (skin) ($\times 10^5$)	$\frac{t_{(soln)}}{t_{(skin)}}$ ($\times 10^3$)
0.06	4.23	4.02	1.05
0.12	2.12	1.68	1.26
0.25	1.06	0.62	1.71
0.50	0.53	0.41	1.29

[a] Transference number in solution is calculated from the fraction of current carried by anodal current.

(The mobility of sodium ion in solution used in the calculation is 5.2×10^{-4} cm$^2 \cdot$s/Coulomb).

for the two solution compartments. Besides, the mobility of the ions in the skin is expected to be much less than that in the solutions. The only competing cationic species present in the donor solution is sodium ion; therefore, Eqn. 4 can be reduced to:

$$t_{AVP(anodal)} = \frac{C_{AVP} \cdot z_{AVP} \cdot u_{AVP}}{C_{AVP} \cdot z_{AVP} \cdot u_{AVP} + C_{Na^+} \cdot z_{Na^+} \cdot u_{Na^+}} \quad (5)$$

where $t_{AVP(anodal)}$ is the fraction of anodal current carried AVP in solution.

By using Eqn. 5, $t_{AVP(anodal)}$ in solution at different ionic strengths can be calculated (Fig. 6). The results indicate that $t_{AVP(anodal)}$ in solution decreases as the ionic strength of the donor solution increases.

The transference number of AVP in the skin under an applied current density, I, can be expressed as:

$$t_{AVP} = \frac{z_{AVP} \cdot F \cdot J_{AVP}}{\Sigma I} \quad (6)$$

where J_{AVP} is the flux of AVP (nmol/cm^2/h) and the unit of I is mA/cm^2.

The transference numbers of AVP in the solution and in the skin at different ionic strengths are compared in Table 4. The magnitude of these values reflects the degree of ease of AVP transport in the solution and across the skin.

Effect of AVP concentration. The effect of donor AVP concentration on the skin permeation flux of AVP in the presence of iontophoresis application is summarized in Table 5. The results indicate that an increase in AVP concentration in the donor solution produces a proportional increase in AVP flux. A similar finding was also reported by Bellantone and her co-workers (1986). The data suggest an 8-fold increase in AVP concentration causes about a 9-fold enhancement in AVP flux at an ionic strength of 0.25 M. Furthermore, as the ionic strength is reduced to 0.06 M, the AVP flux is enhanced by up to 16-fold. The

TABLE 5

Effect of concentration of arginine-vasopressin on its steady-state iontophoresis-facilitated permeation rate [a]

Concentration (mM)	Skin permeation rate [b] (nmol/cm^2/h ± S.D.)	
	Study A	Study B
0.01	0.055 (±0.011)	0.166 (±0.050)
0.02	0.122 (±0.022)	0.414 (±0.097)
0.04	0.220 (±0.029)	1.30 (±0.14)
0.08	0.509 (±0.056)	2.71 (±0.25)

[a] At a current density of 0.47 mA/cm^2.
[b] With ionic strength of:
Study A = 0.25 M.
Study B = 0.06 M.

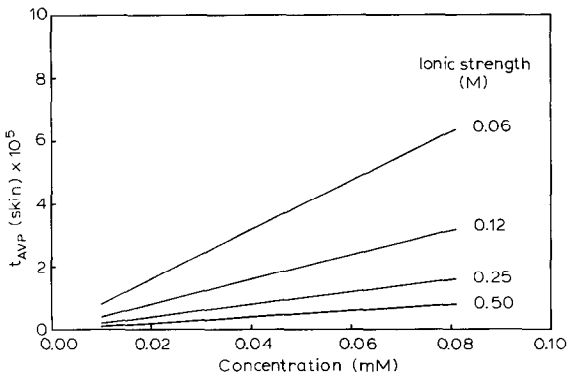


Fig. 7. Effect of concentration of arginine-vasopressin in the donor solution on its calculated membrane transference number (t_{AVP}) at different ionic strengths.

effect of AVP concentration in the donor solution on $t_{(skin)}$ at different ionic strengths can be predicted from $t_{(soln)}$ using the known ratio of $t_{(soln)}/t_{(skin)}$ which is approximately 1.33 (average value obtained from Table 4). The plot of calculated $t_{(skin)}$ for vasopressin at different concentrations for various ionic strengths is shown in Fig. 7.

This suggests that the enhancement in the iontophoretic transport of AVP can be optimized. Although AVP flux was observed to increase as the AVP concentration increased, this relationship was not so significant at high ionic strength. A balance between the AVP concentration and the ionic strength of the donor solution is necessary in order to optimize formulation design.

The AVP flux, J, during the iontophoretic process can be described by the following equation:

$$J = J_p + J_e \tag{7}$$

where J_p = passive diffusion flux of AVP; J_e = electrical-facilitated flux of AVP. Or

$$J = -D\left[\frac{dC}{dx} + C \cdot \frac{d \ln \gamma}{dx} + \frac{zF}{RT} \cdot a \cdot \frac{dV}{dx}\right] \tag{8}$$

where γ = activity coefficient of AVP in the donor solution; a = activity of AVP; dV/dx = electrical potential gradient across the skin.

During iontophoresis, the skin permeability might be temporarily altered by an expansion of the existing channels or a creation of new pores (Burnette and Marrero, 1986; Burnette and Ongpipattanakul, 1988), the fluidization of lipid matrix in the intercellular spacings by the heat and hydration (Knutson et al., 1985), or the rearrangement of proteins in the stratum corneum. An increase in the mobility of charged molecules in the skin also leads to an increase in the diffusion coefficient. As a result, the diffusion coefficient of the drug ions under iontophoresis might not be constant.

The contribution of convective flow, such as electro-osmosis, has been reported to have a great impact on the in vitro iontophoresis-facilitated skin permeation of drugs (Burnette and Marrero, 1986; Burnette and Ongpipattanakul, 1987). Skin is considered as a negatively-charged membrane when exposed to a solution with pH greater than 3–4. The electro-convective flow of water across the skin has been observed to increase as the solution pH is increased (Rein, 1924). One may expect that as the density of negative charge in the skin is increased by increasing the pH of the solution, so the convective flow of positive ions will also be increased. However, no results in this series of studies has demonstrated this point.

Upon the termination of current application, the permeation rate of AVP gradually returns to that of passive diffusion (Fig. 8). The time required for AVP flux to return to passive diffusion has been found to be determined at least partly by

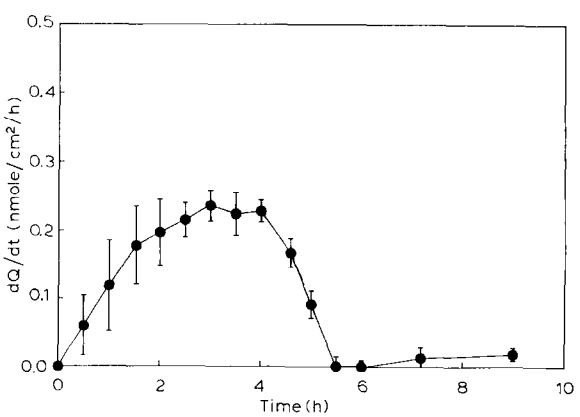


Fig. 8. The time course for the skin permeation flux (dQ/dt) of arginine-vasopressin during and after iontophoresis treatment (current was applied continuously for 4 h).

the time needed for the drug molecules to desorb from the skin (Lelawongs et al., 1989). The flux at the post-treatment period was found reasonably close to the control value. It suggests that no major irreversible alterations in skin permeability occur during iontophoresis.

Conclusions

The enhanced permeation of a model peptide, AVP, through the hairless rat skin has been achieved with the use of iontophoresis. The enhancement in AVP permeation can be optimized by altering the physicochemical conditions of the system. It has been observed that the flux can be substantially enhanced by minimizing the amount of competitive ions present in the donor solution. In addition, a linear relationship was established between the flux of AVP and its concentration at different ionic strengths.

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