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# Transdermal iontophoretic delivery of arginine-vasopressin (II): Evaluation of electrical and operational factors

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## Summary

In vitro iontophoretic delivery of arginine-vasopressin (AVP) across the rat skin was carried out to explore various electrical and operational factors of pulsed D.C. iontophoresis as well as their effect on the enhancement of skin permeation of AVP. The skin permeation rate was found to be a linear function of the density of pulse current and the duration of application. By varying the current input rate, mode of application and buffer capacity, the desirable rate of skin permeation could be obtained. The skin permeation was observed to return to passive diffusion after the termination of iontophoresis treatment. It generally required approx. 1.5–2 h of iontophoresis treatment for AVP permeation to reach steady state. It was demonstrated that AVP can be delivered transdermally by consecutively applying, stopping and reapplying the electric field alternatively. The effect of the frequency of pulse current on the in vitro skin permeation by iontophoresis was found to be negligible and the reason for the observation was analyzed.

## Introduction

Currently, the systemic delivery of most peptide/protein hormones is accomplished via the parenteral route of administration. Since they are extensively degraded by the proteolytic enzymes in the gastrointestinal tract, their bioavailability is extremely poor when taken orally. Furthermore, they are mostly short-acting, frequent injections often being necessary in order to maintain therapeutically useful levels. Over the years, much effort has been directed to the search for a potential route, other than the parenteral route of administration, to deliver peptides and proteins.

Since peptides/proteins are very potent, they must be delivered at a proper dose. The delivery pattern is also critical for the pharmacological activities of some peptide/protein hormones (Banga and Chien, 1988). LHRH and its analogs, for instance, have been shown to have opposite pharmacological effects when administered in a pulsatile or continuous pattern (Cutler et al., 1985). Similarly, it has been reported that pulsatile delivery, rather than steady input, is also required for the proper therapeutic action of vasopressin (Koch and Lutz-Bucher, 1985). Continuous administration of this hormone has been reported to result in the possible development of tolerance or

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'down-regulation', since the maintenance of a steady level at a receptor site can lead to reduction of activity.

Transdermal iontophoresis is one of the techniques which are capable of administering drugs in a pulsatile pattern by alternately applying and terminating the current input at a programmed rate. In addition, the delivery rate can be controlled by the intensity of the electric current applied.

Arginine-vasopressin is a nonapeptide hormone. It has a molecular weight of 1084 (base). Owing to the presence of basic arginine and the blocking of the C-terminus with $-NH_2$, it has an isoelectric point as high as 10.9.

The objective of this series of investigations was to study the effect of electrical parameters, such as current density and frequency, and operation parameters, such as duration of application and dosing pattern, on the iontophoresis-facilitated transdermal delivery of arginine-vasopressin. Results of these investigations are reported in this article.

## Materials and Methods

### *Materials*

All chemicals were used as received. Synthetic arginine-vasopressin (AVP; obtained as the acetate salt, from Sigma, St. Louis, MO) was used. Radiolabelled AVP ([*phenylalanyl*-3,4,5-$^3$H-(N)]AVP; New England Nuclear, Boston, MA) with a specific activity of 70.0 Ci/mmol (purity approx. 99%) was used as a tracer during the in vitro skin permeation studies. Biofluor$^R$ (New England Nuclear) was used as a liquid scintillation cocktail.

Platinum wires (99.95% purity, 1 cm × 0.5 mm, Johnson Mattey, Seabrook, NH) were used to prepare the electrodes in the studies.

### *In vitro iontophoresis skin permeation*

In vitro skin permeation kinetic studies were performed using a full-thickness hairless rat skin (HRS strain, Armed Force Institute of Pathology, Washington, DC), which was freshly excised immediately after killing the animal by injection of Euthanasia solution (0.3 ml/kg). The skin specimen was mounted between the donor and receptor compartments of a Valia-Chien (V-C) skin permeation cell (Crown Glass, Somerville, NJ). The surface area of the skin available for drug permeation was maintained at 0.64 $cm^2$. The donor compartment was filled with 3.0 ml of a freshly prepared solution of unlabeled AVP (at 50 $\mu$g/ml or approx. 0.04 mM) and its radioactive tracer in a citrate-phosphate buffer solution at pH 5.0 with an ionic strength of 0.06 M (except for the buffer capacity study). The receptor compartment was filled with 3 ml of isotonic Sorensen phosphate buffer at pH 7.4 as receptor solution. The solutions in both compartments were stirred by a matched pair of starhead-shaped magnets rotating at 600 rpm and the temperature was maintained at 37 ° C.

A pair of platinum electrodes was immersed in the solutions with the anode in the donor compartment and the cathode in the receptor compartment. These electrodes were connected to the transdermal periodic iontotherapeutic system (TPIS), an adjustable constant-current power source with simple d.c. and pulsed d.c. features. A pulsed d.c. current with square-waveform, a frequency of 2 kHz and on/off ratio of 1 : 1 was used. The electric current was applied either periodically or in a continuous manner. The current, which was applied in a periodic manner, was turned 'on' for 10 min and then turned 'off' for 30 min as one cycle and was applied for 6 or 12 cycles before being terminated. For continuous application, the current was turned on continuously, without interruption, for 4 h.

Samples of 0.5 ml each were withdrawn at predetermined intervals from the receptor solution, which was immediately replaced with a fresh drug-free buffer solution. The samples were mixed with scintillation cocktail and their radioactivity then counted in a liquid scintillation counter (Rack Beta/LKB, Gaithersburg, MD). The amount of drug permeated through the skin was monitored continuously during the course of iontophoresis treatment and then for an additional 5 h after termination of the treatment.

The potential developed across the skin during iontophoretic application was measured using a pair of Ag/AgCl microelectrodes, in a saturated

solution of KCl (Microelectrode, Londonderry, NH), which were positioned directly on the skin surfaces, one on the epidermal side, the other on the dermal side, and connected electrically to a voltage recorder. The potential across the skin was monitored over a period of 1 h. Since the passage of electric current through the skin during the potential measurement lowered the skin resistance, the measurement was not repeated on the same skin. In other words, a fresh skin specimen was used for each potential measurement.

*Purity and identity of radiolabelled AVP*

The purity and identity of radiolabelled AVP were evaluated by thin-layer chromatography (TLC). The solution was spotted on a silica gel plate, previously cut into strips. A 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, approx. 1.5 cm long. The silica gel was scraped off the plate, collected into the vials containing scintillation cocktail and the radioactivity then subsequently measured.

The receptor samples were checked against the standard by the method outlined above at the end of current application. The purity of radiolabelled AVP as determined by TLC, was approx. 70–75%.

*Effect of current density.* The dependence of the steady-state flux of AVP on current density was studied using pulsed d.c. current with a current density in the range 0.47–1.56 mA/cm$^2$ (periodic mode: 6 cycles) and 0.16–0.94 mA/cm$^2$ (continuous mode). A lower range of current was chosen for application in the continuous mode than that in the periodic mode to minimize the damaging effect on the skin specimen.

*Effect of buffer capacity.* In studying the effect of buffer capacity, the buffer capacity of the donor solution used was maintained at 36, 9 and $4.5 \times 10^{-3}$ M/pH unit (ionic strength: 0.24, 0.06 and 0.03 M, respectively). A pulse current at 0.94 mA/cm$^2$ was applied continuously.

*Effect of current input rate.* The effect of current input rate was investigated by either altering the current density or changing the application pattern. In the first study, pulsed d.c. with a current density of 0.078, 0.31 or 0.47 mA/cm$^2$ was applied, in continuous fashion, respectively, for 240, 60 and 40 min. These combinations of current density and application time were designed to keep the total amount of electrical energy applied at a constant level. In the second study, a fixed current density was applied while the current application pattern was varied, either in a periodic or in a continuous manner with the same total duration of current application. For example, a current density of 0.16 mA/cm$^2$ was applied periodically at 10 min/cycle for 12 cycles or continuously for 120 min.

*Effect of application duration.* Pulsed d.c. was applied continuously at a fixed current density (0.47 mA/cm$^2$), while the duration of continuous current application was varied: 20, 40, 60 and 240 min.

*Pulsatile dosing by iontophoresis.* Pulsed d.c. was applied for a duration of 20, 40 or 60 min and the iontophoresis treatment then terminated for about 5 h with continuous sampling. The same procedure of current application was repeated for the second and third dosing.

*Comparison between pulsed d.c. and simple d.c.* Comparative studies were conducted at pH 5.0 using pulse current of different frequencies (0–16 kHz) at a fixed current density of 0.78 mA/cm$^2$. Current was applied in a periodic manner for 6 cycles.

## Results and Discussion

*Effect of current density*

In Fig. 1, the skin permeation profile of arginine-vasopressin (AVP) under iontophoresis treatment is compared with that by passive diffusion. By passive diffusion alone, AVP permeates through the skin at an extremely low rate (0.0008 nmol/cm$^2$ per h). Its permeation rate was significantly enhanced by iontophoresis treatment. The extent of enhancement in skin permeation rate was observed to increase with increasing current density applied either periodically or continuously (Fig. 2).

The magnitude of enhancement in the permeation rates of AVP following iontophoresis treat-

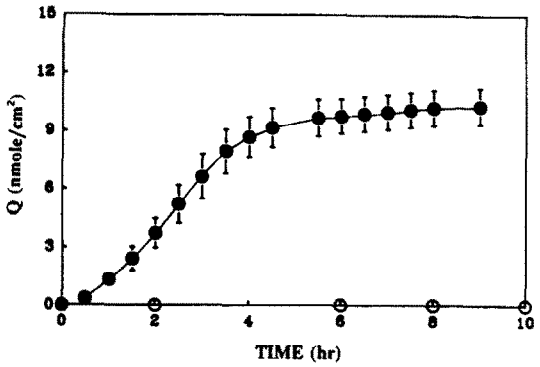


Fig. 1. Comparison of skin permeation profiles of arginine-vasopressin for (○) passive diffusion, and (●) iontophoresis treatment (pulsed d.c., 2 kHz, 0.94 mA/cm$^2$) for 4 h.

ment was dependent not only upon the current density applied, but also on the delivery mode of the applied current. The data in Table 1 indicate that the enhancement in steady-state flux is greater for the current applied in a continuous mode than that with a periodic mode. After correcting for the difference in the total duration of current application, a continuous mode of iontophoresis treatment appears to achieve an enhancement factor

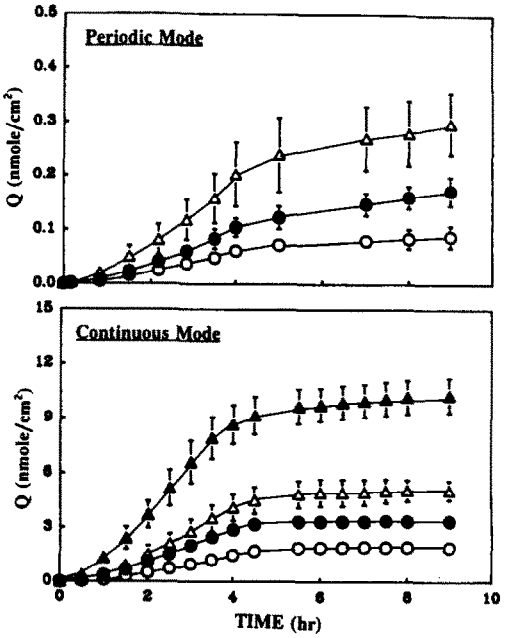


Fig. 2. Effect of current density on the permeation profiles of arginine-vasopressin across hairless rat skin. Pulsed d.c. (2 kHz) of various current densities was applied in either periodic or continuous mode. (Periodic mode) (△) 1.56 mA/cm$^2$, (●) 0.78 mA/cm$^2$, (○) 0.47 mA/cm$^2$. (Continuous mode) (▲) 0.94 mA/cm$^2$, (△) 0.47 mA/cm$^2$, (●) 0.31 mA/cm$^2$, (○) 0.16 mA/cm$^2$.

TABLE 1

*Effect of current density on skin permeation rate of arginine-vasopressin* [a]

| Current density (mA/cm$^2$) | Skin permeation rate (nmol/cm$^2$ per h) | Enhancement factor [b] | |
|---|---|---|---|
| | | Apparent | Corrected [c] |
| Passive diffusion | 0.0008 (±0.0006) | 1.0 | 1.0 |
| *Iontophoretic permeation* | | | |
| Cyclic mode | | | |
| 0.47 | 0.049 (±0.011) | 61.2 | 61.2 |
| 0.78 | 0.102 (±0.009) | 127.5 | 127.5 |
| 1.56 | 0.158 (±0.022) | 197.5 | 197.5 |
| Continuous mode | | | |
| 0.16 | 0.461 (±0.010) | 576.2 | 144.1 |
| 0.31 | 0.880 (±0.069) | 1100.0 | 275.0 |
| 0.47 | 1.272 (±0.138) | 1590.0 | 397.5 |
| 0.94 | 2.950 (±0.251) [d] | | |

[a] Hairless rat was used.

[b] Enhancement factor = $\frac{\text{skin permeation rate (iontophoresis)}}{\text{skin permeation rate (passive)}}$.

[c] Corrected for the 4-fold difference in the duration of current application between periodic mode (6 × 10 min) and continuous mode (240 min).

[d] Peak flux is reported here since the steady state was not reached.

Note: The average post-iontophoretic passive rate was 0.008 (±0.007) nmol/cm$^2$ per h.

which is 6.5-times higher than that of periodic iontophoresis at the same current density, such as at 0.47 $mA/cm^2$.

*Effect of buffer capacity*

In the in vitro skin permeation studies, platinum wires were used as the electrodes. Even though they are known to cause pH shifts and bubbling of gas in solutions, especially when a high current is applied, they do not cause peptide to precipitate as do the Ag/AgCl electrodes. Therefore, it is important to provide the solution used in the iontophoretic device with a sufficient buffer capacity when platinum electrodes are employed. Generally, the solution should be buffered at a particular pH, so that most of the drug molecules are present in ionized forms. In addition, the buffer capacity of the system has to be high enough to accommodate all the hydronium and hydroxide ions produced, due to the electrolysis of water, at the anode and cathode, without significant shift in solution pH. These buffer species, however, are also potential current-competing species due to their greater mobility as compared to that of charged AVP molecules. Hence, it is important to optimize the concentration of buffer species used in the system, which should be sufficiently high to maintain good buffer capacity but should not reach an extent such that the current is mostly carried by the buffer species instead of the drug species. If this occurs, a low efficiency of iontophoresis will result.

To study the effect of hydronium ion produced during the course of iontophoresis on the $dQ/dt$ vs time profile, a donor solution at pH 5.0 was prepared to contain varying buffer capacities (in the range $4.5–36 \times 10^{-3}$ M/pH unit). Buffer capacity is defined as the capacity of a buffer to resist changes in pH. For example, a buffer solution with a buffer capacity of $36 \times 10^{-3}$ M/pH unit will increase or decrease 1 pH unit when 36 mmol of hydroxide or hydronium ion is added to 1 liter of the solution. To minimize pH shifts, the buffer capacity of the receptor solution in this investigation was kept high enough to accommodate all the hydroxide ion produced at the cathode during current application.

As a water molecule is electrolyzed between a pair of platinum electrodes, the following reaction occurs at the anode:

$$H_2O \rightarrow 2H^+ + \tfrac{1}{2}O_2 + 2e^-$$

The reaction suggests that the movement of 1 mol electron is involved in the generation of 1 mol hydronium ion. Therefore, the amount of hydronium ion produced in the donor solution can be computed from a known amount of current applied:

$$[H^+] = 3600\frac{It}{F} \cdot \frac{1000}{3V}$$

where $[H^+]$ denotes the concentration of hydronium ion (M), $I$ the current intensity (mA), $t$ the application time (h), $F$ the Faraday constant (96 500 C/mol) and $V$ is the volume of solution (3 ml).

It was calculated that approx. 30 mM hydronium ion is produced when a current of 0.6 mA is applied for 4 h (volume of donor solution: 3.0 ml). The production of hydronium ion is known to be a linear function of time. Experimentally, it was observed that under this condition, the pH in the solution with the highest buffer capacity ($36 \times 10^{-3}$ M/pH unit) decreased only slightly from 5.0 to 4.25 ($\pm 0.05$), which compares favorably with the calculated value of 4.0. The pH was found to drop to 2.80 ($\pm 0.20$) and 2.22 ($\pm 0.01$) when the buffer capacity in the donor solution was reduced to 9 and $4.5 \times 10^{-3}$ M/pH unit, respectively. At these low pH values, the concentration of hydronium ion is about 1000-times higher than that of AVP ion, resulting in greater competition for the current in the system. Furthermore, the hydronium ion is highly mobile in solution under electric field. It can be transported by a chain transfer mechanism, in which hydronium ion jumps from one water molecule to the next, resulting in a high electric mobility (Borckris and Reddy, 1977). The electric mobility of hydronium ion is approx. 7-times higher than that of sodium ion (Weast, 1985–1986). It has also been reported that the hydronium ion diffuses through biological membranes via the hydrogen bonding of the proteins,

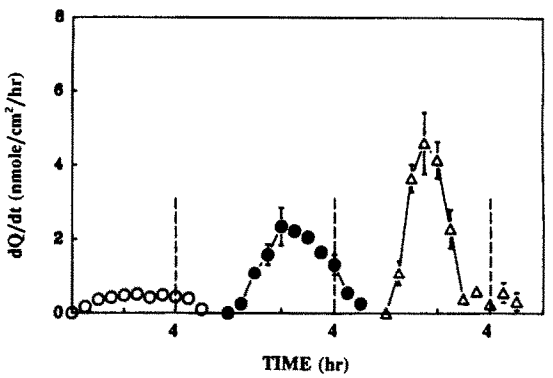


Fig. 3. Importance of buffer capacity of donor solution on the time course for the permeation rate of arginine-vasopressin ($dQ/dt$). The buffer capacity of the receptor solution was kept constant and sufficient. Pulsed d.c. (2 kHz) at a density of 0.94 mA/cm$^2$ was applied continuously for 4 h. (○) buffer capacity = $36\times10^{-3}$ M/pH unit, (●) buffer capacity = $9\times10^{-3}$ M/pH unit, (△) buffer capacity = $4.5\times10^{-3}$ M/pH unit.

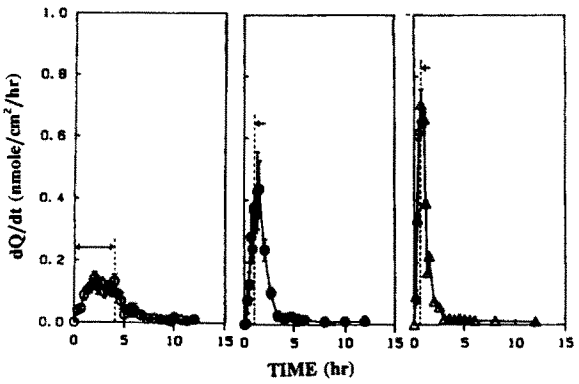


Fig. 4. Time course for skin permeation rate ($dQ/dt$) of arginine-vasopressin at different current input rates. Pulsed d.c. (2 kHz) was applied continuously. (○) 0.078 mA/cm$^2$ for 240 min, (●) 0.31 mA/cm$^2$ for 60 min, (△) 0.47 mA/cm$^2$ for 40 min.

and hence, its transport can be even more accelerated (Nagle and Morowitz, 1978).

The skin permeation rate profiles in Fig. 3 demonstrate that a steady-state permeation rate of AVP could be achieved by maintaining sufficient buffer capacity in the system. However, as the buffer concentration increases, the amount of applied current available to the AVP ion will decrease, which yields a low AVP flux. In contrast, for systems having a lower concentration of buffer species, the flux of AVP is significantly increased but steady-state permeation is unattainable. This results from a gradual increase in the transference number of hydronium ions in the system.

*Effect of current input rate*

Fig. 4 shows the time course for AVP flux ($dQ/dt$) when the current was applied at 0.078, 0.31 and 0.47 mA/cm$^2$, respectively, for 240, 60, and 40 min, in a continuous mode. The results indicate that the peak AVP flux increases with the increase in current density. The cumulative amount of AVP permeated through the skin was found to be significantly higher following the iontophoresis treatment with high current density, despite the fact that in all three cases, the same amount of electric energy (Coulombs) had been applied.

Fig. 5 compares the time course for the skin permeation rate ($dQ/dt$) of AVP facilitated by pulsed d.c. applied in two different modes, continuous and periodic, at the same current density. As shown in Table 1, the results again demonstrate that periodic iontophoresis produces a lower flux than the continuous one. This is possible due to the fact that the maximum permeation rate of AVP is never reached during 10-minute current application. It is interesting to note that under periodic iontophoresis, AVP molecules continu-

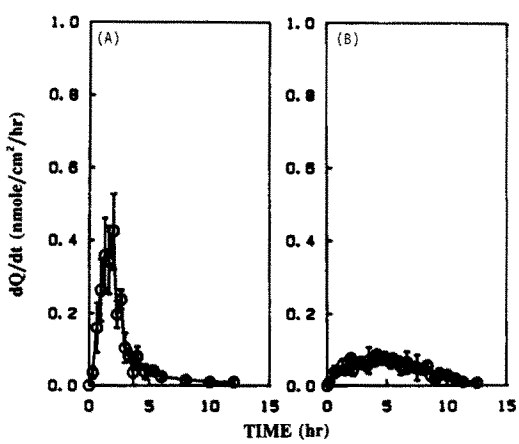


Fig. 5. Comparison of skin permeation rate ($dQ/dt$) profile of arginine-vasopressin for different modes of iontophoresis treatment. Pulsed d.c. (2 kHz) was applied. (A) 0.16 mA/cm$^2$ (continuous application: 120 min), (B) 0.16 mA/cm$^2$ (periodic application: 12 cycles, 10 min each).

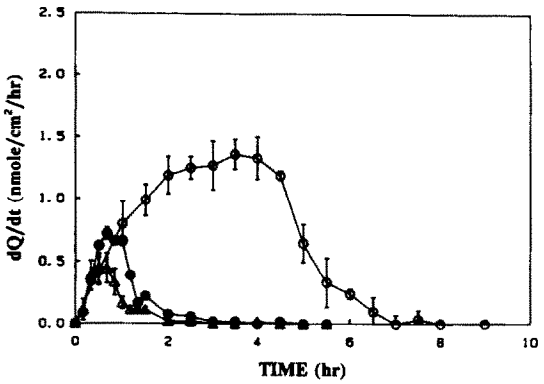


Fig. 6. Skin permeation rate ($dQ/dt$) profile of arginine-vasopressin for different durations of iontophoresis treatment. Pulsed d.c. (2 kHz) at a density of 0.47 mA/cm$^2$ was applied. (○) Application time = 240 min, (●) application time = 40 min, (△) application time = 20 min.

ously permeate through the skin even after terminating the current intermittently. This observation might be due to the existence of drug reservoir in the skin.

*Effect of duration of application*

The effect of the duration of iontophoresis treatment on skin permeation rate of arginine vasopressin is shown in Table 2. As expected, the total amount of drug transported through the skin

TABLE 2

*Effect of duration of current application on skin permeation of arginine-vasopressin*

| Duration of application [a] (min) | Total amount permeated [b] (nmol/cm$^2$) (±SD) |
|---|---|
| 20 | 0.43 (±0.07) |
| 40 | 0.80 (±0.08) |
| 60 | 1.22 (±0.07) |
| 240 | 5.08 (±0.32) |

[a] A current density of 0.47 mA/cm$^2$ was applied continuously for the duration of application specified.

[b] The cumulative amount of arginine-vasopressin permeated through hairless rat skin up to the plateau region.

is linearly increased with increasing application time.

The skin permeation rate ($dQ/dt$) profiles in Fig. 6 suggest that the longer the duration of application, the higher the plateau rate of permeation. It is of interest to note that the time taken to reach the steady-state permeation rate for AVP is approx. 2 h. When the current was terminated prior to this time point, steady-state flux was not achieved.

*Pulsatile dosing by iontophoresis*

The transdermal delivery of AVP by iontophoresis in pulsatile pattern is shown in Fig. 7A. It is

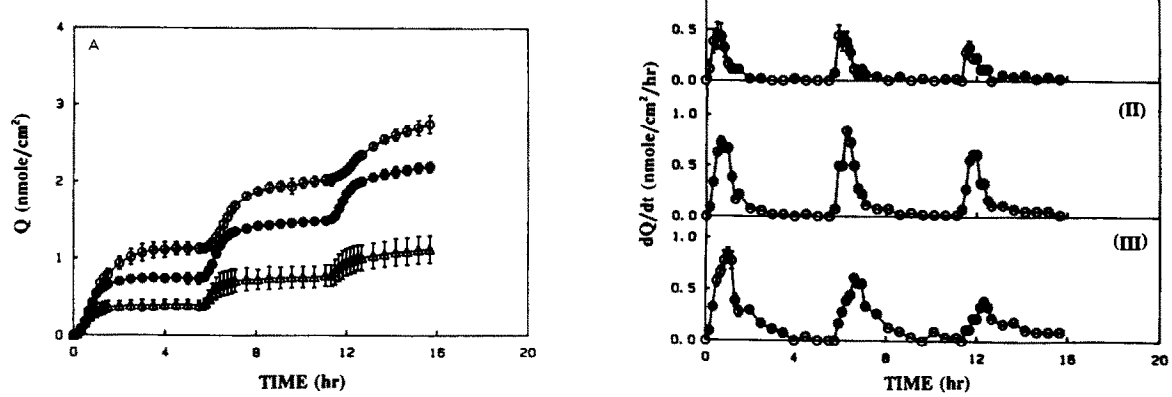


Fig. 7. (A) Skin permeation profiles of arginine-vasopressin following pulsatile dosing by iontophoresis. Pulsed d.c. (2 kHz) at a current density of 0.47 mA/cm$^2$ was applied periodically. (○) 60 min/application, (●) 40 min/application, (△) 20 min/application. (B) Skin permeation rate profiles of arginine-vasopressin following pulsatile dosing by iontophoresis. Pulsed d.c. (2 kHz) at a current density of 0.47 mA/cm$^2$ was applied periodically. (I) 20 min/application, (II) 40 min/application, (III) 60 min/application.

TABLE 3

*Cumulative amount of arginine-vasopressin permeated through the skin at each current application*

| | Total amount permeated [a] (nmol/cm$^2$) (±S.D.) |
|---|---|
| Group I (20 min/application) | |
| 1st Application | 0.39 (±0.06) |
| 2nd Application | 0.38 (±0.07) |
| 3rd Application | 0.35 (±0.08) |
| Group II (40 min/application) | |
| 1st Application | 0.74 (±0.02) |
| 2nd Application | 0.76 (±0.04) |
| 3rd Application | 0.74 (±0.07) |
| Group III (60 min/application) | |
| 1st Application | 1.01 (±0.01) |
| 2nd Application | 0.93 (±0.07) |
| 3rd Application | 0.79 (±0.07) |

[a] The cumulative amount of arginine-vasopressin permeated through hairless rat skin up to the plateau region.

noted that the cumulative amount of AVP permeated through the skin is a function of the duration of iontophoresis treatment for the first two treatments and maintains a rather constant level throughout all three treatments for a duration of treatment of less than 40 min, but not for 60 min (Table 3). The data in Fig. 7B indicate that the skin permeation rate increases during the iontophoresis treatment and gradually decreases following current termination (Fig. 7B). It should be pointed out that steady-state flux was not achieved because the current was applied for only a short period of time (< 2 h, Fig. 6). The skin permeation rate attained in the post-treatment phase (following the termination of iontophoresis treatment) was found to be reasonably close to that achieved by passive diffusion alone.

*Comparison between pulsed d.c. and simple d.c.*

The results obtained from the in vitro iontophoretic transdermal permeation studies at various frequencies, ranging from 0 to 16 kHz, showed no significant difference in the steady-state flux of AVP at both pH 5 and 7.4 (Fig. 8). The permeation rate of AVP across the skin during current application at pH 7.4 was lower than that for pH 5; this is due to a greater ionic strength of the

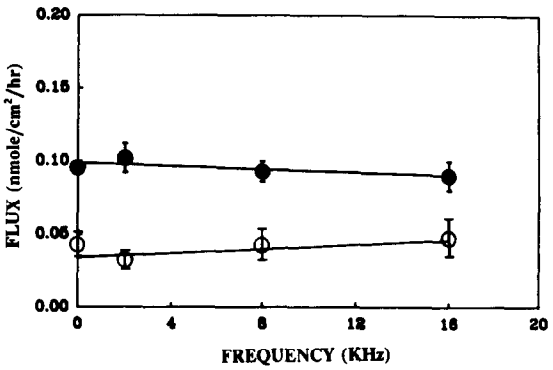


Fig. 8. Effect of the frequency of pulsed d.c. on the steady-state permeation rate of arginine-vasopressin. Current density of 0.78 mA/cm$^2$ was applied in a periodic manner for 6 cycles. (●) pH 5, (○) pH 7.4.

system used at pH 7.4. The ionic strength of the buffer solution at pH 7.4 was 0.50 M while that of pH 5 was 0.25 M. The studies on the effect of variation in the current density on the skin permeation rate of AVP also revealed no statistical difference in the flux between simple d.c. and pulsed d.c. at a frequency of 2 kHz.

The following discussion is a speculative one, however, it might provide some explanation for our experimental results.

The electrical property of skin can be described by a simple equivalent circuit as shown in Fig. 9. $R_2$ denotes the pure resistance originating from the viable skin, which is very small and does not change with frequency. Usually, it lies within the range 0.1–1.0 kΩ (Tregear, 1966). The parallel combination of $R_1$ and C represents the resistor and capacitor of the impedance in the stratum corneum. These resistive and capacitive components are observed to change with frequency (Yamamoto and Yamamoto, 1988). Theoretically,

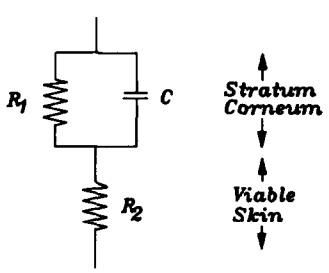


Fig. 9. Equivalent electric circuit of the skin.

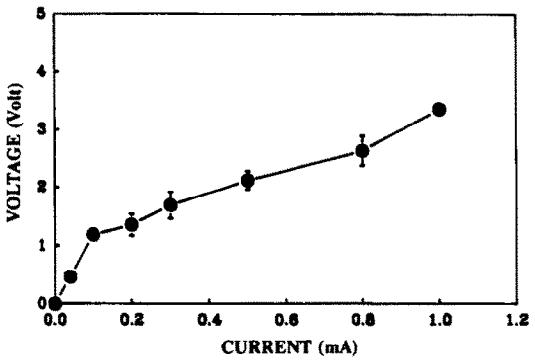


Fig. 10. Current-initial voltage relationship of freshly excised rat skin.

the skin impedance will fall as a result of capacitive shunting as the frequency is raised. When the direct current is applied continuously to the skin, the polarization impedance develops toward infinity and the current passes mainly through the pathway consisting of $R_1$ and $R_2$. On the other hand, as a pulse current is applied at a very high frequency, the polarization impedance approaches zero, as a result of depolarization process, and the impedance of the system is close to $R_2$. The skin permeation of drug by iontophoresis might be enhanced as the skin impedance is lowered at high frequency.

Our result on the electrical properties of excised skin suggested that the skin shows non-linear behavior when the applied current exceeded a current intensity of around 0.10 mA (Fig. 10). This observation suggests that beyond this region, the skin impedance does not follow Ohm's law, indicating that the skin impedance decreases to a greater extent as a higher current is applied. In addition, the skin impedance was also observed to change with the duration of iontophoresis treatment (Fig. 11). A rapid decrease in skin impedance occurs in the first 10 min of current application, and thereafter the skin impedance value was almost at a steady level, i.e., approx. 1–3 k$\Omega$ (per 0.64 cm$^2$) with pulsed d.c. at a current density of 0.78 mA/cm$^2$. The higher the pulse current applied, the lower the skin impedance. These phenomena resulted in a significant decrease in the $R_1$ value and might have obscured the effect of frequency on the capacitive component. Also, the effect of frequency on the resistive component is minimal as $R_1$ has such a low value (Yamamoto and Yamamoto, 1988). This might be responsible for the observed absence of an increase in the in vitro skin permeation rate of AVP with the increase in frequency.

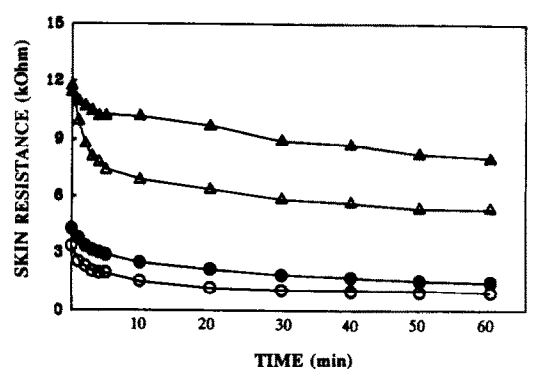


Fig. 11. The time course for the change in skin resistance during current application. (▲) 0.04 mA, (△) 0.1 mA, (●) 0.5 mA, (○) 1.0 mA.

## Conclusion

The results generated in this series of investigations have demonstrated that, with the assistance of iontophoresis, the transdermal delivery of arginine-vasopressin has been substantially facilitated over the permeation by passive diffusion. By proper optimization of the electrical and operational parameters, arginine-vasopressin can be delivered through the intact skin at a controlled rate and with desirable dosing pattern.

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