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Transdermal Iontophoretic Delivery of Thyrotropin-Releasing Hormone Across Excised Rabbit Pinna Skin

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ABSTRACT

Enhanced transport of a model peptide drug thyrotropin-releasing hormone (TRH), a tripeptide of molar mass 362 g and a pK_a 6.2, through excised rabbit pinna skin was achieved by means of iontophoresis with continuous current or monophase periodically pulsed current. The resulting flux in the steady state was proportional to the applied current density. In the transdermal iontophoretic delivery of TRH, the pulsed iontophoretic flux exceeded that obtained with a continuous current. At a low ionic strength, an increased degree of protonation in TRH increased the rate of permeation. The flux of TRH in a buffer at pH 4 is greater than that at pH 8 when the ionic strength is 0.1 M. At a greater ionic strength, the trend is reversed. The enhanced flux of unprotonated TRH during transdermal iontophoresis is attributed largely to electro-osmotic volume flow. An increased rate of permeation of TRH crossing the skin is achieved at low ionic strength, moderate pH, and a large duty cycle of current; the frequency of pulsed current had no significant influence on the rate of transdermal iontophoretic delivery of TRH.

INTRODUCTION

Peptide/protein drugs have become increasingly important in medication as a result of both our understanding of their role in physiology and pathology and the rapid advances in genetic engineering and biotechnological process (1). They are active pharmacologically and highly specific. Only a minute dose is needed at each treatment. These substances cannot be administered orally and are thus therapeutically effective only on parenteral administration. Most peptides have short biological half-lives, and repeated injections are typically

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required to maintain their therapeutic efficacy. Such injections cause patients anxiety and pain.

Transdermal delivery is an attractive method for peptide delivery (2) because it is noninvasive, avoids chemically hostile gastrointestinal environment, and avoids hepatic elimination at first pass. It also provides a controlled and sustained delivery. The major difficulty is that skin is so excellent a barrier that it prevents transport of large molecules and hydrophilic compounds. Unfortunately, most therapeutic peptides and proteins are charged, large, or hydrophilic, or have all these properties.

Iontophoresis can enhance the transdermal delivery of charged permeants. Charged molecules and ionic drugs, even neutral molecules, can be driven increasingly through skin into the body under an electrical potential, in either continuous mode or pulsed mode (3,4). Another advantage of delivery by this mode is to administer the drug in a pulsatile manner, which is critical for the desired physiological response of some hormonal drugs (5,6).

Thyrotropin-releasing hormone (TRH), a hypothalamic regulatory hormone, is a small peptide being used widely in research on peptide delivery. It has the structure Glp-His-ProNH_2, a weakly basic tripeptide with a molar mass of 362.42 *g* and pK_a 6.2. In this work, we investigated the feasibility of enhancement of transdermal delivery of TRH facilitated by iontophoresis with continuous current or by monophase periodically pulsed current, and possible ways to enhance and to control the transdermal delivery of other peptides.

MATERIALS AND METHODS

TRH (Sigma Chemical Company, St. Louis, MO, USA) and other chemicals (reagent grade, Sigma Chemical Company or Wako Chemicals, Japan) were used as received unless otherwise stated. Water was obtained from a purification system (Milli-Q, Millipore Inc., Miliford, MA, USA).

Determination of Iontophoretic Flux In Vitro

The basic experimental arrangement is depicted in Fig. 1. Iontophoretic diffusion experiments in vitro were made in the Valia-Chien side-by-side diffusion cell (model VSC-1, Crown Glass, Somerville, NJ), with a controlled rate of stirring (600 rpm) in both half-cells. An isothermal condition at 37 ± 0.2°C was maintained in both half-cells by circulating water of controlled temperature through a water jacket. An orifice (0.69 cm^2) connected the two diffusion cells. The volume of each cell was 3.5 ml. Platinum wires (99.99% purity; length 2 cm × diameter 0.5 mm) served as electrodes. The electrodes were connected to a universal power source (HP 3245A, Hewlett-Packard, Palo Alto, CA), which output a periodically pulsed or constant current.

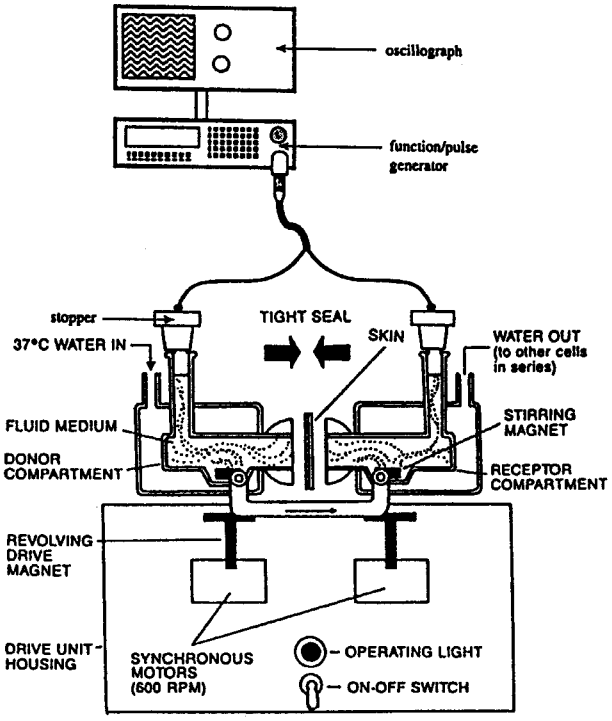

Figure 1. Experimental arrangement for transdermal iontophoretic delivery in vitro of TRH.

Skin was mounted between diffusion cells. In the donor side, TRH (1 mg/ml) was loaded in the donor buffer. TRH permeated from the donor side through the skin to the receptor compartment. To simulate the body fluid, a Sorensen phosphate buffer (pH 7.4) was used as the receiver solution. TRH permeates from the donor side through skin to the receptor side. Phosphate buffer was also used in donor solution, but the pH and buffer capacity were adjusted with weak acid and NaOH (0.1 N) to meet the requirements of each experiment. In some cases, phosphate-citrate buffer was also used in an acidic environment as the donor solution. For comparison with results of Burnette and Marrero (7), phosphate-citrate buffer (pH 4), consisting of Na_2 HPO_4 (0.083 M), citric acid (0.059 M), NaCl (0.427 M); and phosphate-citrate buffer (pH 8), consisting of Na_2 HPO_4

(0.195 M), citric acid (0.0028 M), NaCl (0.041 M) were also used.

Rabbit inner pinna skin was used in all experiments as its transdermal permeation characteristics are similar to human cadaverous skin (8). Skin was freshly excised from New Zealand white rabbits, about 3–5 kg, obtained from our animal center in the College of Medicine. The outermost layers of skin (epidermis skin) were taken from the animals immediately after they were sacrificed and were used as soon as possible.

Determination of TRH

TRH was assayed by capillary zone electrophoresis (P/ACE System 2100, Beckman Instruments, Fullerton, CA). The capillary cartridge contained a capillary of fused silica of length 57 cm (50 cm to detector) and internal diameter 75 μm. An ultraviolet (UV) detector at 214 nm was used, and the temperature of the capillary was maintained at 30 ± 0.1°C. The separation buffer contained phosphate (pH 7.4) at an ionic strength 0.02 M. Before each injection, the column was preconditioned by flushing, firstly, with NaOH (0.1 M) for 1 min, secondly, with deionized water for 1 min, and finally, with separation buffer for 2 min. The sample was injected into the column under pressure for 5 sec, corresponding to approximately 30 nl. All separations were performed for 10 min at a constant voltage 15 kV. The retention time of TRH was about 7.55 min. The sample signals were analyzed with software (Beckman System Gold, version 8.1).

RESULTS AND DISCUSSION

In electrochemistry, a silver/silver chloride electrode is commonly used for various purposes because of its small emf that can prevent electrolysis of water. In permeation tests in vitro, this electrode caused serious precipitation of TRH when an external electrical potential was applied. Platinum electrodes are preferred to deliver peptides with iontophoresis, but this inert electrode resulted in a shift of pH and gas bubbling from electric hydrolysis of water and concomitant production of H^+ and OH^- ions.

For TRH, undetectable amounts of drug are transported across excised skin during passive diffusion (without iontophoresis). Figure 2 shows the effect of applied current on transdermal delivery of TRH. With continuous DC iontophoresis and a citrate buffer (pH 4.0), the rate of permeation of TRH increased with respect to the applied current. The cumulative amount of TRH was linearly increased as duration of iontophoresis increased. According to linear regression, the cor-

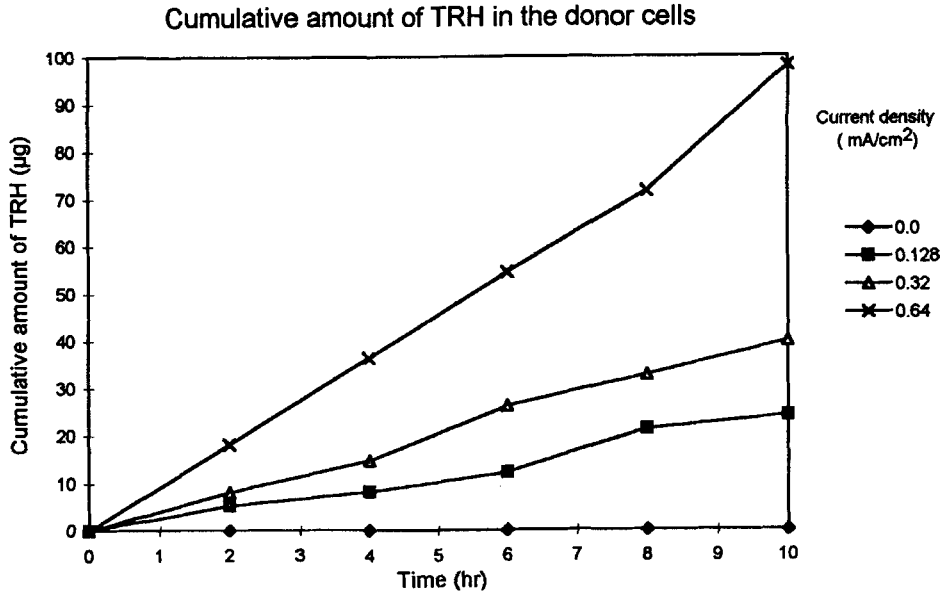

Figure 2. Cumulative amount of TRH in the receptor cell versus iontophoretic duration at varied applied current density. Donor (3.5 mg/ml) in citrate buffer (pH 4.01); receptor, phosphate buffer (pH 7.4).

relation coefficient is $r = 0.968$. Increased current density during iontophoresis clearly elicits augmented permeation of TRH. Flux of TRH increased linearly with applied current density under the same experimental conditions.

The flux of TRH, J_i, is attributed to three mechanisms of transport—passive diffusion, electrically induced motion of ions, and electro-osmotic convection, represented in Eq. (1).

$$J_i = J_{ip} + J_{ie} + J_{iv}$$

$$J_i = D\nabla C_i - \frac{Dz_iFC_i}{RT}\nabla\phi + J_{iv} \quad (1)$$

As experimental results show that J_{ip} approaches zero, and according to Pikal's model, $J_{iv} = L_{ve}\nabla\phi$, the flux is proportional only to the applied electrical field, $\nabla\phi$.

$$J_i = -\frac{Dz_iFC_i}{RT}\nabla\phi - L_{ve}\nabla\phi \quad (2)$$

Experimental results of Kim et al. (9) showed that the resistance of the skin barrier soon approaches a steady value, as shown in their Fig. 1. As a result, the flux of TRH is directly proportional to the total applied current density. Figure 3 shows the flux of TRH versus applied current density, I_d. According to linear regression, the flux of TRH, Y (μg/hr·cm²) = 19.9734 × I_d (mA/cm²); R^2 is 0.9832.

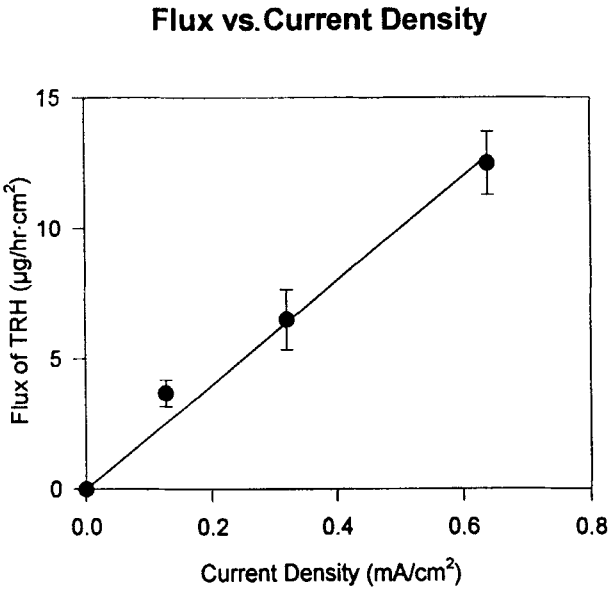

Figure 3. Plot of flux of TRH versus current density; the flux of TRH (μg/hr·cm²) = 19.9734 × I_d (mA/cm²), R^2 is 0.9832.

Most peptide drugs have different degrees of protonation depending on pH of the bulk solution. At pH 8, TRH is 98% unprotonated. With the buffer at small pH, the ratio of protonated TRH increases and attains 99% protonated ion at pH 4. The charge and degree of dissociation of drug affect the effectiveness of iontophoresis. A greater degree of protonation intuitively provides a greater rate of permeation. Results shown in Fig. 4 confirm this hypothesis. Flux of TRH in a buffer at pH 4 exceeds that at pH 8. Burnette and Marrero (7) showed that unprotonated TRH at pH 8 had a greater flux of permeation than protonated TRH at pH 4 under the same current density. They explained that property to be due partly to TRH being transported by convection to a greater extent at pH 8. In their experiments, the ionic strength of the buffer (0.6 M) was too great to impede the drug from moving. Buffer ions are primary carriers of charge. Only <1% of total charge is transported by protonated TRH during iontophoresis. They proposed convection as the major contribution to the mechanism of transport in their system. If the ionic strength of the buffer solution were smaller, the competing ionic species became fewer. The drug would have a greater possibility of being driven by applied electrical potential. In contrast, a lower ionic strength would diminish the flow of electro-osmosis and convection. The best combinations of ionic strength and pH need further investigation.

When the ionic strength is decreased to 0.1 M, the flux of TRH in a buffer at pH 4 exceeds that in a buffer at pH 8. The trend is reversed (Fig. 4). When the ionic strength was 0.1 M, the flux (15.05 μg/cm²) of TRH in a buffer of pH 4 exceeded that (8.29 μg/cm²) in a buffer at pH 8. Other than pH of the donor buffer, ionic strength is a key factor in operating transdermal delivery of a peptide drug.

Another concern in transdermal iontophoretic delivery of TRH is the kind of current, continuous or pulsed, for iontophoresis, which can improve the efficiency of transport. Although Bagniefski and Burnette (3) showed that pulsed iontophoretic flux of Na^+ was equal to the flux obtained from an equivalent continuous current, in transdermal iontophoretic delivery of TRH, the pulsed iontophoretic flux exceeded that obtained with continuous current (Fig. 5). An electrical field with iontophoresis in a continuous mode may cause an electrochemical polarization in the skin that impedes the drug from pen-

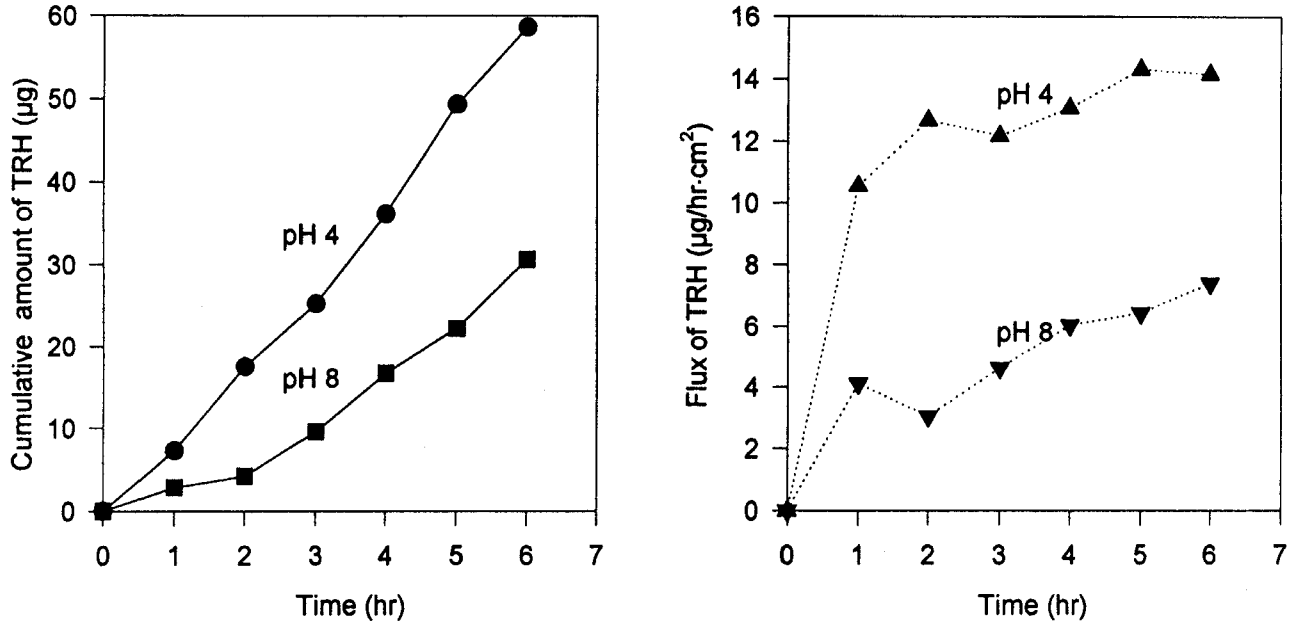

Figure 4. Flux of TRH at pH 4 and pH 8, ionic strength 0.1 M, under iontophoresis.

etrating. Depolarization of the skin during iontophoresis with pulsed current resulted in a significantly increased amount of transdermal delivery of TRH.

Other than pH and ionic strength, frequency of pulsed current, amplitude, and the duty cycle of pulsed current are the most important parameters to influence the rate of permeation of peptide/protein through skin under iontophoresis. Varied ratios on/off of the electric device in a pulsed mode cause varied degrees of skin polarization that inhibit the drug from permeating through the skin.

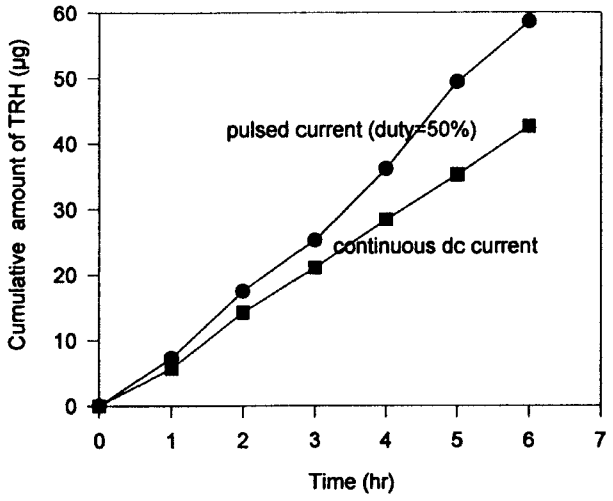

Figure 5. Cumulative amount of TRH in the receptor cell versus iontophoretic duration with continuous and pulsed current.

Table 1 shows the conditions of eight experiments and their cumulative amount of transdermal permeation of TRH during iontophoresis with a pulsed current for 6 hr. The flux of TRH was varied according to operating conditions. Donor solution with a small ionic strength (μ = 0.05, open symbols in Fig. 6) yields a greater amount of permeation than with a large ionic strength (μ = 0.15, closed symbols in Fig. 6). The variation was 7.5–52 μg/hr cm²—an almost sevenfold variation. A small pH also increases the flux because of a large portion of TRH protonated. The flux of TRH, Y (μg/hr cm²), was obtained according to the linear least squares method.

$$Y = 20.9 - 2.3x_1 - 12.0x_2 - 0.1x_3 + 8.0x_4 \quad (3)$$

in which x_1 represents the pH of the donor buffer, x_2 represents the ionic strength of the donor buffer, x_3 represents the frequency of the applied pulsed current, and x_4 is the duty cycle of the applied pulsed current.

Equation (3) indicates that ionic strength is the most important parameter to control the transdermal iontophoretic permeation of TRH. Each variation of ionic

Table 1

Experimental Conditions and Permeation Flux in Iontophoretic Transdermal Delivery of TRH

Factor	pH	Ionic Strength (M)	Frequency (Hz)	Duty Cycle (%)	Flux ($\mu g/hr/cm^2$)
Exp. 1	6.5	0.15	3000	60	7.9991
Exp. 2	6.5	0.15	300	40	7.5298
Exp. 3	6.5	0.05	3000	40	13.1642
Exp. 4	6.5	0.05	300	60	45.4571
Exp. 5	5.5	0.15	3000	40	9.8890
Exp. 6	5.5	0.15	300	60	9.8404
Exp. 7	5.5	0.05	3000	60	52.0100
Exp. 8	5.5	0.05	300	40	20.9346

strength by 0.05 M caused variation by 12 $\mu g/hr\ cm^2$ of permeation flux of TRH. The second most important factor is duty cycle. In this batch of experiments, the amplitude of current was maintained constant. With a large duty cycle, much work is done by the external electrical field; hence more ions are carried through the tissue membrane by electrical potential. The importance of pH on the flux of permeation of TRH is -4.6 $\mu g/hr\ cm^2$ per unit pH variation.

CONCLUSIONS

Iontophoresis is a promising method for enhancement of permeation of drugs through the skin. Transport of a model peptide drug (TRH) through excised rabbit pinna skin was enhanced with iontophoresis either by continuous current or monophase periodically pulsed current. The resulting flux in the steady state was proportional to the applied current density. In the transdermal iontophoretic delivery of TRH, pulsed iontophoretic flux exceeded that obtained with continuous current. The effects of pH, ionic strength in the donor compartment, and duty cycle on the drug transport are important in operating this system.

With an increased ionic strength in the donor buffer, an enhanced flux of unprotonated TRH during transdermal iontophoresis is attributed largely to electroosmotic volume flow. As a low ionic strength, greater protonation of TRH increased the rate of permeation. A greater rate of permeation of TRH crossing the skin was achieved at a small ionic strength, moderate pH, and large duty cycle of pulsed current; but the frequency of pulsed current had no significant influence on the rate of transdermal iontophoretic delivery of TRH.

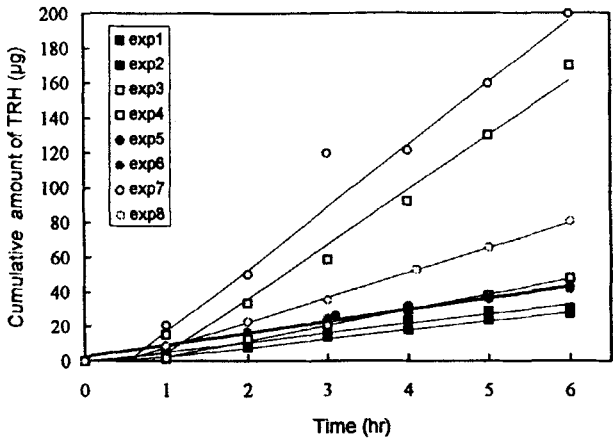

Figure 6. Cumulative amount of TRH in the receptor cell versus iontophoretic duration at various operating conditions with respect to Table 1 under iontophoresis.

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