Transdermal Delivery of Metoprolol by Electroporation

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Electroporation, i.e., the creation of transient "pores" in lipid membranes leading to increased permeability, could be used to promote transdermal drug delivery. We have evaluated metoprolol permeation through full thickness hairless rat skin in vitro following electroporation with an exponentially decaying pulse. Application of electric pulses increased metoprolol permeation as compared to diffusion through untreated skin. Raising the number of twin pulses (300 V, 3 ms; followed after 1 s by 100 V, 620 ms) from 1 to 20 increased drug transport. Single pulse (100 V, 620 ms) was as effective as twin pulse application (2200 V, 1100 V or 300 V, 3 ms; followed after 1 s by 100 V, 620 ms). In order to investigate the effect of pulse voltage on metoprolol permeation, 5 single pulses (each separated by 1 min) were applied at varying voltages from 24 to 450 V (pulse time 620 ms). A linear correlation between pulse voltage and cumulative metoprolol transported after 4 h suggested that voltage controls the quantity of drug delivered. Then, the effect of pulse time on metoprolol permeation was studied by varying pulse duration of 5 single 100 V pulses from 80 to 710 ms (each pulse also separated by 1 min). Cumulative metoprolol transported after 4 h increased linearly with the pulse time. Therefore, pulse time was also a control factor of the quantity of drug delivered but to a lesser extent than the voltage at least at 100 V. The mechanisms behind improved transdermal drug delivery by electroporation involved reversible increased skin permeability, electrophoretic movement of drug into the skin during pulse application, and drug release from the skin reservoir formed by electroporation. Thus, electroporation did occur as shown by the increased transdermal permeation, on indicator of structural skin changes and their reversibility. Electroporation has potential for enhancing transdermal drug delivery.

KEY WORDS: transdermal drug delivery; electroporation; metoprolol; skin permeation.

INTRODUCTION

Transdermal drug delivery is a useful alternative to the conventional routes of administration such as oral or injectable routes. It avoids degradation in the gastrointestinal tract and first-pass hepatic metabolism. Transdermal administration allows steady or time varying controlled delivery and improves patient compliance. However, very few drugs can be administered transdermally due to the low permeability of the skin. It is presently applicable for only a few drugs. Such molecules are typically small and relatively lipophilic. For charged, polar molecules, administration by the cutaneous route is difficult due to the intrinsic lipophilicity of the stratum corneum. Several methods have been employed to enhance transdermal delivery of such drugs (1). Chemical penetration enhancers have been extensively studied. Iontophoresis is another technique used to enhance delivery of charged and neutral polar molecules. It uses an electrical potential gradient as a driving force. However, if iontophoresis offers the possibility of systemic delivery in a controlled and effective fashion without extensive skin damage (1,2), it needs low intensity electric current application for several minutes if not hours and is limited to relatively low molecular weight drugs.

Electroporation is a phenomenon in which the membranes of cells or lipid bilayers exposed to high intensity electric field pulses are temporarily destabilised and permeabilized. In recent years, it has been recognized as a powerful method of transporting macromolecules, such as DNA or proteins, into cells. In spite of this success, the basic mechanisms of electroporation remain largely unknown. It is generally accepted, however, that the application of an electric pulse may create transient aqueous pores in the cell membrane (3).

The barrier properties of the skin are attributed primarily to the stratum corneum, the skin's outermost layer. The stratum corneum is composed of flattened dead cells filled with keratin and intercellular matrix with multilamellar lipid bilayers. It has been suggested that electroporation of the skin could promote transdermal drug delivery by creating transient changes in tissue permeability consequent to the "electroporation" (creation of transient aqueous pores) of the stratum corneum's intercellular lipid bilayers (4–10). While iontophoresis acts primarily on the drug, involving skin structural changes as a secondary effect, electroporation is expected to act directly on the skin making change in tissue permeability. Thus, occurrence of skin electroporation should be associated with increased transport, reversibility and evidence for skin structural changes (9).

The aim of this study was to investigate the influence of electroporation on the in vitro transdermal permeation of metoprolol, a β blocker with a high first pass hepatic metabolism (pKa = 9.7, MW = 267, $K_{oct/water}$ = 0.6) (11). The influence of the pulses' number, pulse type, pulse voltage, pulse time were studied in vitro using hairless rat skin. The mechanisms of transport by electroporation were also examined.

MATERIALS AND METHODS

Chemicals

(±)-Metoprolol (+)-tartrate and *dl*-(−)-propranolol hydrochloride were purchased from Sigma Chemical Company (St. Louis, MO). The salts used to prepare the buffers (for analysis), methanol (HPLC grade) and acetonitrile (HPLC grade) were obtained from UCB (RPL, Leuven, Belgium). All solutions were prepared in ultrapure water (Sation 900, Vel, Leuven, Belgium).

In Vitro Model

The in vitro model was a polycarbonate (Makrolon,

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Obra, Liège, Belgium) horizontal cell made of two chambers separated by a membrane. The surface area of the membrane exposed to the two solutions was 3 cm^2. The upper (donor) compartment contained 1.6 ml of drug solution. The receptor compartment with a capacity of 7.5 ml was continuously stirred magnetically and maintained at 37°C. A pair of platinum electrodes (platinum pure, Johnson Matthey, Brussels, Belgium) of 1 cm^2 were immersed in the solutions (if not mentioned, the anode was in the donor compartment and the cathode in the receptor compartment). The distance between each electrode was approximately 1 cm.

The experiments were carried out with male hairless rat skin (mutant Iops rat hairless; Iffa Credo, St Germain les Arbreles, France). The 2-3-month-old rats were sacrificed by ether breathing, and full-thickness abdominal skin was excised. Subcutaneous fat was removed carefully with scissors. The freshly excised skin specimens were mounted between the two half-cells of the permeation system, with the stratum corneum facing the donor compartment.

The receptor compartment was filled with a phosphate buffer (0.024 M) at pH 7.4 isotonized with glucose (0.151 M). Metoprolol tartrate (10 mg/ml) was introduced in phtalate buffer (0.01 M) at pH 3, resulting in a final pH of 4. No shift in pH due to pulsing was observed. Samples of solution (0.3 ml) were taken from the receptor compartment at regular intervals (0.5 or 1 h) up to 4 h after the pulses, and were replaced with an equal volume of the drug free buffer. When kinetics of permeation was followed, dilution of receptor compartment was taken into account. All samples were frozen until analyzed (11). The ratio of the cumulative quantities detected in the receptor compartment to the membrane area was plotted in term of time. The lag times were deduced from the linear part of the plot when it was possible. The results were expressed as means ± the standard error of the means (n = 3 to 6).

Electroporation

The electrodes are connected to Easyject Plus® (Equibio, Seraing, Belgium), an equipment used for electroporating bacteria and other cell membranes. Easyject Plus® is an electroporation system based on capacitor discharge. The voltage can vary from 3500 V to 20 V and is an exponentially decaying capacitive discharge pulse. Pulse time is defined as the time constant i.e. as the length of time between the beginning of the pulse (maximum voltage) and the time when the voltage reaches 37% of its maximal value. This pulse length is measured by Easyject Plus®. It depends on the electrical circuit resistance (composed of the shunt resistance of Easyject Plus® and of the apparent cell diffusion resistance) and the capacity of the electroporation apparatus: pulse time = resistance × capacity. The electroporation system Easyject Plus® allows to modify the pulse time by modifying the "timing resistance" and the capacity of the electroporation device.

Easyject Plus® can generate "high voltage" (HV) pulse from 3500 V to 100 V with a maximum capacity of 25 μF and/or a "low voltage" (LV) pulse from 450 V to 20 V and a capacity varying from 150 to 3000 μF, the resistance varying from 99 Ω to infinity in both modes. High voltage (HV) pulses of 2200, 1100 or 300 V were generated with a 25 μF capacity and 329 Ω resistance. Low voltage (LV) pulses of 450, 400, 350, 300, 250, 200, 150, 100, 74, 50 or 24 V were generated with a 2310 Ω resistance and a 3000 μF capacity to get a pulse time as long as possible. Easyject Plus® can be programmed to generate either single pulse (HV or LV) or twin pulse consisting in a first HV pulse, and interpulse delay (1 s) and a second LV pulse. If more than one pulse was applied, they were separated by 1 min. Voltages are expressed as applied values and not transdermal values.

During a pulse, the apparent resistances of the diffusion cell, of the solutions (resistance of the cell without skin but filled with donor or receptor solutions) and then of the skin were evaluated. They were calculated by taking into account the pulse length, the capacity and the shunt resistance of Easyject Plus® and, the distance between each electrode and the skin. Transdermal voltages were evaluated by calculating the ratio of the apparent skin resistance to the apparent total cell resistance. This ratio is equal to the ratio of the transdermal voltage to the voltage across the whole diffusion cell (applied voltage) (9).

Analysis of Metoprolol

The drug concentration was determined by a reversed-phase high-performance liquid chromatographic method. A column of octadecylsilane (μBondapak C_{18}; 30 cm × 3.9 mm; Millipore, Waters, Brussels, Belgium) was used. The mobile phase was methanol:water (adjusted to pH 3 with H_3PO_4):acetonitrile 40:40:20, (v/v/v). The flow rate was 1 ml/min, and 10 μl aliquots were injected. Propranolol (5 μg/ml) was used as the internal standard. UV detection was performed at 222 nm (11,12).

Statistical Analysis

The pulse times, lag times and ratio of cumulative quantities detected in the receptor compartment to the membrane area were compared by the Student t-test ($p < 0.05$).

The kinetics of drug permeation were compared by a two way analysis of variance (Scheffé F-test, $p < 0.05$).

RESULTS AND DISCUSSION

1. Pulses' Number

The influence of the electrical pulses' number on the transdermal passage of metoprolol was first studied. Therefore, a twin pulse composed of a first pulse of 300 HV and a second pulse of 100 LV was used. This twin pulse was applied 1, 5, 10, 15 or 20 times and every twin pulse was separated by 1 min.

The average pulse times of the first HV pulse and second LV pulse were 3.1 ± 0.1 ms and 621 ± 39 ms respectively. The resistance of the diffusion cell decreased from 17 ± 2 kΩ before the pulse to approximately 199 and 227 Ω respectively during the pulse; whereas skin resistance decreased from 16.5 ± 2 kΩ to approximately 74 and 72 Ω respectively.

As shown in fig. 1, application of the electric field pulse strongly enhanced the transdermal permeation of metoprolol as compared to the passive diffusion. It has already been reported that electroporation can achieve significantly ele-

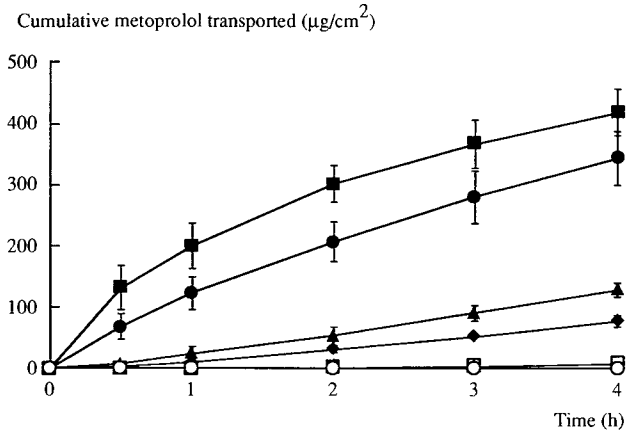

Fig. 1 Plot of cumulative quantities (μg/cm²) of metoprolol vs time (h) after 0 (○), 1 (□), 5 (♦), 10 (▲), 15 (●) or 20 (■) twin pulses: 300 HV (C = 25 μF, R = 329 Ω, 3.1 ms) followed after 1 s by a 100 LV (C = 3000 μF, R = 2310 Ω, 621 ms).

vated fluxes of other molecules using either single, multiple or repetitive pulses of a few tens to a few hundreds volts (4–10).

An increase in the pulses' number was associated with a non proportional increase in cumulative quantities of metoprolol detected in the receptor compartment (Fig. 1). Fig. 2 also confirmed that an increase from 1 to 5 pulses resulted in a non proportional increase in metoprolol permeation. Fig. 1 showed that an increase from 1 to 20 pulses decreased the lag time, and appeared to saturate metoprolol transdermal passage. The instant fluxes between 0 and 30 min after 15 or 20 pulses (136 ± 40 and 261 ± 71 μg/cm².h respectively) returned to lower values between 3 and 4 h (65 ± 5 and 50 ± 9 μg/cm².h respectively) suggesting that changes in skin permeability are reversible.

Fig. 1, 3a, 4, 5 and 6 indicate that even though one pulse lasts less than 1 s, the cumulative quantity of the drug in the receptor compartment increased with time for at least 4 h. However, no average fluxes could be calculated since cumulative quantities did not always increase linearly with time.

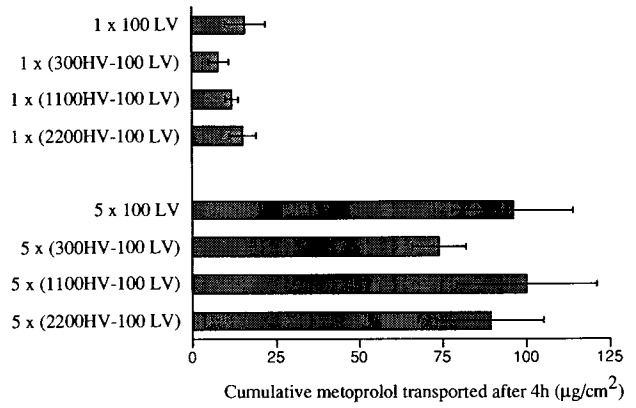

Fig. 2 Cumulative quantities of metoprolol (μg/cm²) transported in the receptor compartment 4 h after the application of 1 or 5 twin pulse(s) (2200 HV-100 LV, 1100 HV-100 LV, 300 HV-100 LV) or single pulse(s) (100 LV) (HV pulse: C = 25 μF, R = 329 Ω, 3.1 ms, followed after 1 s by a LV pulse: C = 3000 μF, R = 2310 Ω, 621 ms).

2. Twin Pulse vs Single Pulse

Easyject Plus® (Equibio) can generate either single pulse or twin pulse. According to Klenchin et al (13) first high voltage pulse could create a set of electropores and start the electrophoretic drift movement; the second low voltage pulse could maintain this movement and lengthen the life of the electropores.

In order to compare the efficiency of twin pulse and single pulse, the permeation of metoprolol was evaluated after 1 or 5 twin pulses (300 HV, 1100 HV or 2200 HV; 3 ms followed after 1 s by a LV pulse 100 V, 620 ms) or 1 or 5 single LV (100 V; 620 ms) pulses application.

As shown in fig. 2, no significant differences were observed when the voltage of the first HV pulse varied from 300 to 2200 V for both 1 or 5 twin pulses (t-test). Therefore, a single LV pulse equivalent to the second pulse of the twin pulse was applied. This single pulse was as efficient as the twin pulse to promote metoprolol permeation, indicating that twin pulse application was not necessary. This long pulse (621 ± 39 ms) at a low voltage was much more efficient than a high voltage pulse with a short pulse time (3.1 ± 0.1 ms) to

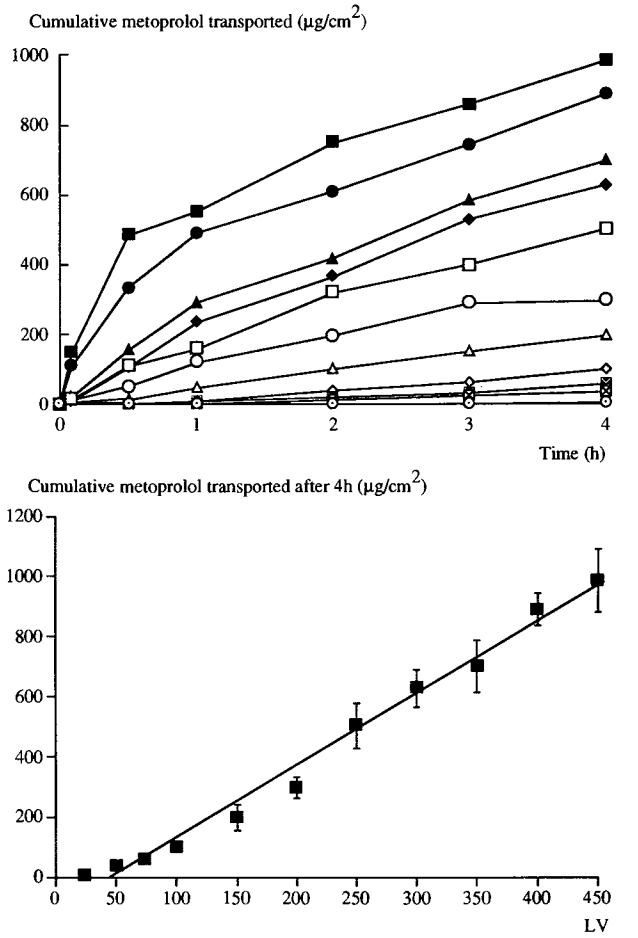

Fig. 3 3a. Plot of the cumulative quantities of metoprolol (μg/cm²) vs time (h) after 5 single LV pulses of 24 V (⊙), 50 V (⊠), 74 V (⊠), 100 V (◇), 150 V (△), 200 V (○), 250 V (□), 300 V (♦), 350 V (▲), 400 V (●), or 450 V (■) (C = 3000 μF, R = 2310 Ω). 3b. Plot of cumulative quantities of metoprolol after 4 h (μg/cm²) vs voltage of the 5 LV pulses applied.

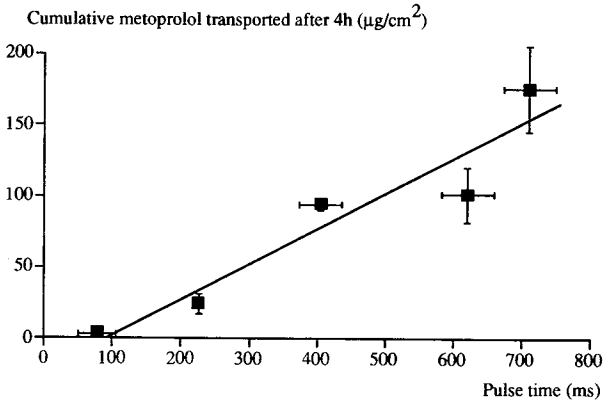

Fig. 4 Plot of the cumulative quantities of metoprolol transported in the receptor compartment (μg/cm²) 4 h after 5 single 100 LV pulses with different pulse times: 78 ± 27 ms obtained with C = 300 μF and R = 2310 Ω; 226 ± 3 ms obtained with C = 3000 μF and R = 99 Ω; 405 ± 31 ms obtained with C = 1950 μF and R = 2310 Ω; 621 ± 39 ms obtained with C = 3000 μF and R = 2310 Ω; and 711 ± 38 ms obtained with C = 3000 μF and R = ∞.

promote metoprolol permeation (cumulative metoprolol transported 4 h after the application of 5 300 HV pulses = 1 μg/cm²) suggesting that pulse duration could be a critical factor to control drug permeation.

The short high voltage pulses used in this study hardly had any effect, while pulses of hundreds of volts and a few ms time constants were reported to have dramatic effects on transdermal permeation (6–10). But, the authors have used human epidermis which is more impermeable (skin resistance equal to 10^5 Ω.cm²) than rat skin (10^4 Ω.cm²) and other experimental conditions. Bommannan et al (7) have applied an exponentially-decaying voltage pulse (transdermal voltage ranging from 300–400 V and pulse time of 5–9 ms) followed by iontophoresis with a constant current or a constant voltage for 10 to 60 min; while Prausnitz et al (9) have applied an exponential-decay pulse (transdermal voltage around 100 V and pulse time of 1 ms) every 5 s for 1 h.

3. Voltage

In order to check whether the voltage of the pulse could influence the transdermal permeation of metoprolol, 5 single LV pulses (2310 Ω, 3000 μF, 620 ms) with a voltage 24, 50, 74, 100, 150, 200, 250, 300, 350, 400 or 450 V were applied; the corresponding transdermal voltages were only ±25% of these values. We noticed that the 400 and 450 V pulses generated effervescence and heat.

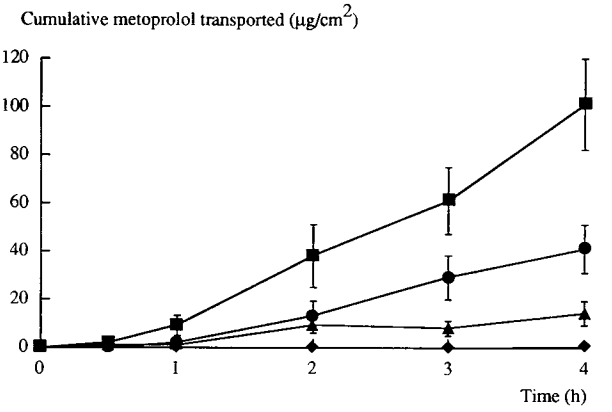

Fig. 5 Plot of the cumulative quantities of metoprolol (μg/cm²) vs time (h) after passive diffusion (0 V) (♦), or 5 single 100 LV pulses (C = 3000 μF, R = 2310 Ω, 621 ms) with the anode (■) or cathode (▲) in the donor compartment or, with NaCl (0.15 M) (●) added to the donor compartment.

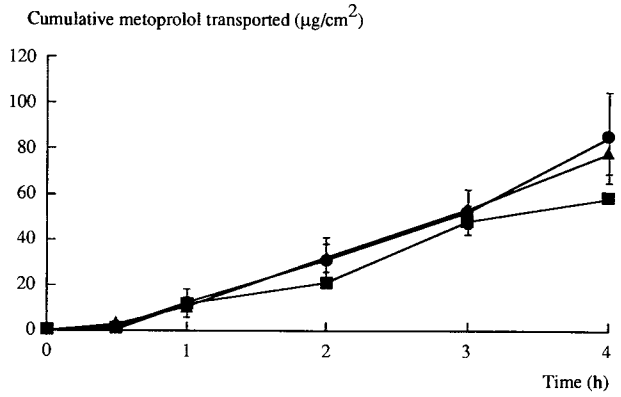

Fig. 6 Plot of cumulative quantities of metoprolol (μg/cm²) vs time (h) after 5 twin pulses (300 HV-100 LV) (for details see legend of fig. 1). The donor containing solution in the donor compartment was replaced by a drug free buffer after 0 h (immediately after pulses) (■), 1 h (●) or 4 h (▲).

Fig. 3a shows that when the voltage increased the cumulative quantities detected in the receptor compartment increased and, also that the lag time dropped progressively from approximately 2 h (24 LV) to 0 h (400 and 450 LV). The pulse time was slightly longer than 620 ms for the 74, 50 and 24 V pulses, inversely, for the voltage higher than 200 V, this pulse time was a bit shortened. This showed that the pulse time and then the skin resistance drop during the pulse was dependent of the pulse voltage. This pulse time increased slightly between the first and the fifth pulse. As for the study of the pulses' number's influence, the comparison of instant fluxes of metoprolol at the beginning and the end of the experience shows the reversibility of skin electroporation. For instance, after the application of the 250 and 450 V pulses, the fluxes between 0 and 30 min, 218 ± 81 and 968 ± 244 μg/cm².h respectively, returned between 3 and 4 h to 104 ± 26 and 126 ± 24 μg/cm².h respectively. However, these instant fluxes remained constant between 1 h and 4 h after the low voltage pulse applications (<200 V).

Fig. 3b indicates that the quantities of metoprolol permeating through the skin after 4 h increased linearly with the voltage indicating that the voltage drop of the pulse could allow to control transdermal administration by electroporation, at least in the experimental conditions used. Prausnitz et al (6) reported that the inverse of the minimum transtissue impedance appeared to vary linearly with applied pulse voltage, suggesting that "electroporation" or electropermeation of the skin is voltage dependent.

4. Pulse Time

We then studied the influence of the pulse time on transdermal permeation of metoprolol. Indeed, the pulse time can be increased by enhancing the timing resistance or the capacity of Easyject Plus®. We followed metoprolol permeat-

ing through the skin after the application of 5 single 100 LV pulses (each separated by 1 min) with duration of 78, 226, 405, 621 and 711 ms. As shown in fig. 4, the cumulative quantities of metoprolol transported after 4 h increased linearly with the pulse time; so, the pulse time can also, but to a lesser extent than the voltage (at least at 100 LV), control drug permeation.

We shortened to 25 s and lengthened to 15 min the time duration between the five 100 LV (620 ms) pulses and then compared the resulted kinetics of metoprolol permeation to the "classical" protocol where this time is worth 1 min. We observed that the kinetics are identical when this interpulse duration is 1 min or 15 min but the kinetic is slightly higher when it is worth 25 s (data not shown).

5. Mechanisms of Transdermal Permeation

The mechanism(s) by which electroporation of the stratum corneum's multilamellar intercellular lipid bilayers enhancing transdermal drug delivery might very likely be similar to what has been reported for other bilayer lipid membranes or cells, namely the reversible permeation of the lipid bilayers involving the creation of transient aqueous "pores" by application of the electric pulse (3). However, the electrophoretic drift of the drug by electroporation could also contribute to drug transport (13). Indeed, in the previous experiments, the anode was placed in the donor compartment in which metoprolol was positively charged. Moreover, a "long" pulse application (620 ms) seemed more efficient than a "short" (3 ms) high voltage pulse. In order to check if electrostatic repulsion contributes to drug transport, the electrode polarity was reversed and, in an other experiment NaCl (0.15 M) was added to the donor compartment before application of 5 100 LV pulses (620 ms).

As shown in fig 5, the cumulative quantities of metoprolol in the receptor compartment decreased when the cathode was placed instead of the anode in the donor compartment. It also decreased when competitive ions were added (Na^+) ($p < 0.05$ F-test). However, the amount of drug permeating the skin was always much higher than with passive diffusion (no pulse) ($p < 0.05$ F-test). These results clearly indicate that electroporation increases skin permeability and induces skin structural changes whatever electrodes polarity was chosen. They demonstrate that the role of electrophoretic movement by electrostatic repulsion in transdermal permeation by electroporation is important.

A second set of experiments was designed to assess the reversibility of the permeation and to check whether metoprolol could form a depot in the skin. Therefore, after application of 5 twin pulses (300 HV-100 LV), the donor compartment was replaced by a drug free buffer immediately after or 1 h after pulses application.

As shown in fig. 6, the cumulative quantities of the drug transported in the receptor compartment were not significantly different after 4 h ($p > 0.05$ t-test). However, the permeation kinetics were lower when the drug was immediately withdrawn ($p < 0.05$ F-test). It suggests that the change in skin permeability is rapidly reversible. Other reports tend to support that skin membrane alterations due to electroporation are reversible within several minutes (6,9) or hours (7). Moreover, the results demonstrate that the skin is loaded in vitro at least with metoprolol during electroporation and that the drug is slowly released from the skin reservoir.

6. Evidence for Skin Electroporation

Finally, it is necessary to establish under the experimental conditions used that electroporation, rather than high voltage iontophoresis, has indeed occurred. Besides increased permeability, skin structural changes and skin reversibility should be detectable (9).

Our data demonstrate that electroporation has occurred. Indeed, transdermal transport of metoprolol is clearly increased by pulsing protocol as compared to the diffusion through untreated skin (to 10^3 fold increase) (Fig. 1–6).

The evidence for structural skin changes is more difficult to obtain. However, the increased transport due to pulsing with the "wrong" polarity as compared to diffusion through untreated skin supports structural changes (Fig. 5). Moreover, skin resistance decreased dramatically during pulsing also confirming that skin structure was modified.

Since metoprolol solution can be removed from the donor compartment without greatly decreasing metoprolol transport, the increase in skin permeability induced by pulsing was considered to be rapidly reversible. In addition, after 15 or 20 pulses and after high voltage (250–450 V) pulses, instant fluxes between 3 and 4 h decreased strongly as compared to instant fluxes immediately after pulsing (0–30 min) (Fig. 1 and Fig. 3a). Moreover, when interpulse duration decreased from 15 min or from 1 min to 25 s, metoprolol permeation increased, indicating that transient changes in skin are induced by pulse.

CONCLUSION

Electroporation of skin lipid bilayers could create pores capable of allowing extensive transport of drugs or macromolecules (4–10). This report confirms that the application of electrical pulses can indeed promote transdermal delivery of metoprolol in vitro. We used an in vitro model with full thickness abdominal hairless rat skin as a membrane to evaluate the basic parameters affecting metoprolol permeation in standard conditions (11,14,15). In this model, one or multiple (up to 20) applications of a single "long" (620 ms) low voltage (24–350V) were adequate for enhancing drug permeation. We also showed that the control of the pulses' number, pulse voltage and pulse time allowed to control the quantity of drug delivered through the skin (Fig. 1, 3b and Fig. 4). The mechanisms of transdermal metoprolol delivery by electroporation involved both a transient increase in permeability and an electrostatic repulsion. A drug reservoir was also generated in the skin, even though pulses are very short (Fig. 5–6).

Electroporation may be safe for clinical use under appropriate conditions. In vitro studies indicated that the increase in skin permeability is rapidly reversible (see results; 6, 7, 9). Preliminary experiments in which LV pulses were applied to anesthetized rats did not show any apparent skin alterations nor systemic toxicity (data not shown). However, Prausnitz et al (8) observed occasional erythema and edema. Mir et al (16) and Titomorov et al (17) applied high intensity electric impulsions to mice without any lethality or impor-

tant skin damage. Electrochemotherapy i.e. the application of electric pulses (100 μs, 1300 V cm^{-1}) in combination with chemotherapy has been well tolerated by patients (18).

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