Transdermal Delivery of Fentanyl by Electroporation II. Mechanisms Involved in Drug Transport

Rita Vanbever,[1] Nathalie De Morre[1] and Véronique Préat[1,2]

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Purpose. The aim of the present report was to systematically analyze the mechanisms involved in fentanyl transdermal transport by skin electroporation.

Methods. The study was performed *in vitro* with full-thickness hairless rat skin, skin electroporation being carried out with five exponentially-decaying pulses of 100 V applied voltage and around 600 ms pulse duration.

Results. Transport during and after pulsing are both important in transdermal delivery of fentanyl by skin electroporation. Rapid transport occurred during pulsing due to electrophoresis and diffusion through highly permeabilized skin. No electroosmosis was observed. The slow post-pulse passive transport was explained by lasting changes in skin permeability. Measurements of fentanyl quantities in the skin demonstrated that pulses rapidly loaded the viable part of the skin with fentanyl and hence rapidly overcame skin barrier.

Conclusions. The different contributions of the transport mechanisms appear to depend on the physicochemical parameters of the transported molecule as well as the solution, suggesting that mechanistic analysis and careful consideration of formulation variables are essential for the development and optimization of drug delivery by skin electroporation.

KEY WORDS: transdermal drug delivery; fentanyl; electroporation; transport mechanisms.

INTRODUCTION

Recent developments in pharmaceutical technology are paving the way towards the introduction of novel routes of drug administration which will have a direct and an indirect effect on care practice. Among these approaches, transdermal delivery appears particularly promising because of the accessibility, noninvasiveness, compliance, safety and efficacy associated with the technique. However, due to the multilamellar intercellular lipid bilayers of the stratum corneum (SC), human skin has great barrier properties and only a few drugs typically small, lipophilic, uncharged and potent are clinically delivered (1).

Fentanyl is a synthetic narcotic widely used as an analgesic and an anesthesic agent because of its rapid onset, short duration of action and high potency. It is the prototypical opioid for transdermal application. Fentanyl is a basic (pKa = 8.9) lipophilic (log $D_{oct/water\ at\ pH\ 7.4}$ = 2.86) molecule of a molecular weight of 336 Da (2). A fentanyl transdermal delivery system has been developed for the management of moderate to severe chronic pain (Duragesic®, Janssen). When compared to oral delivery, the sustained release of the transdermal system avoids the extensive first-pass hepatic metabolism. When compared to the bolus injection, it avoids the possible toxic plasma levels and the rapid plasma level decrease due to the short half-time of fentanyl (1.5–6 h). However the important lag time to reach a plateau (14 h) and the lack of opportunity to modulate the rate of the release are important disadvantages of this delivery system (3).

It has been shown that the transdermal transport of small dyes i.e. calcein and sulforhodamine or, drugs i.e. metoprolol and fentanyl can be enhanced by up to four orders of magnitude by application of high-voltage pulses (4–9). Using this approach the transport through human epidermis of even larger drugs such as peptides, antisense oligodesoxyribonucleotides and heparin occurred at significant level (10–12). The phenomenon underlying enhancement is hypothesized to be electroporation of SC lipid bilayers. Electroporation involves the application of high-voltage pulses which create transient aqueous pathways in cell membranes or lipid bilayers and permit local transport of molecules through these pathways (13).

Our previous report (9) evaluated the potential of electroporation in fentanyl transdermal delivery. More specifically it focused on the influence of the electrical parameters of the pulses. Fentanyl permeation through hairless rat skin *in vitro* was shown to be strongly promoted by electrical pulses as compared to passive diffusion through untreated skin. The choice of the waveform of the pulses was important: at the same energy, a few exponentially-decaying pulses increased fentanyl permeation more than a few square-wave pulses and to the same extent as the repeated application of higher voltage-shorter duration exponentially-decaying pulses. Moreover the control of the quantity of drug transported through the skin was achieved by controlling the voltage, duration and number of exponentially-decaying pulses.

Mechanistic insights in transdermal drug transport by electroporation has generally been incompletely investigated, however its comprehension is essential for the optimization of drug delivery. The aim of the present report was to systematically analyze *in vitro* the mechanisms involved in fentanyl transport enhanced by high-voltage pulses through hairless rat skin. We considered four different mechanisms of transport. Three modes of transport could exist during the application of pulses: ion-electric field interaction (electrophoresis), convective flow (electroosmosis), and diffusion possibly enhanced by electroporation-induced increase in skin permeability. After pulsing, only diffusion could occur since no electric field is present. The relative importance of each of these four transport mechanisms was assessed along with the evaluation of the electroporation-induced drug reservoir within the skin. The measurement of fentanyl quantities according to skin depth was also performed (14–15).

MATERIALS AND METHODS

The experimental methods have been fully described previously (5–7,9,15).

Chemicals

Fentanyl citrate was purchased from Janssen Pharmaceutica (Beerse, Belgium). ^{3}H fentanyl was a generous gift from

[1] Université Catholique de Louvain, Unité de Pharmacie Galénique, Industrielle et Officinale, Avenue E. Mounier, UCL 73.20, 1200 Brussels, Belgium.

[2] To whom correspondence should be addressed.

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Janssen Biotech (Olen, Belgium). The salts used to prepare the buffers (for analysis) were supplied by UCB (RPL, Leuven, Belgium). All solutions were prepared in ultrapure water (Sation 9000, Vel, Leuven, Belgium).

In Vitro Model and Procedures

Skin electroporation was performed *in vitro* in up-and-down oriented chambers. 3 cm^2 of freshly excised abdominal hairless rat skin (mutant rat Iops hairless from Iffa Credo, Saint Germain les Arbresles, France) separated the donor and receiver compartments. Platinum electrodes were immersed in the solutions and connected to the electroporation device. Unless otherwise noted, the anode was in the donor solution and the cathode was in the receiver solution as fentanyl is +1 charged at pH 5. The upper reservoir was filled with 1.5 ml of the donor solution: fentanyl (40 μg/ml) citrate and ^3H fentanyl (0.5 μCi/ml) were introduced unless otherwise noted in a citrate buffer at pH 5 0.01 M. The receiver compartment (7.5 ml) was filled with a phosphate buffer (0.024 M) at pH 7.4 made isotonic with glucose, continuously stirred magnetically and maintained at 37°C.

After skin electroporation, fentanyl transport was followed for 6 h by taking samples (0.4 ml) from the receiver compartment at regular intervals. Fentanyl quantities in the samples were measured by β counting. The ratio of the cumulative quantities detected in the receiver compartment to the skin area was plotted in term of time (9). The permeation study was performed with fentanyl solution present in the donor compartment during and after pulsing or, only during pulsing or, only after pulsing. Removing fentanyl solution after pulsing was carried out 1 min after the last pulse by rinsing and filling the donor compartment with the receiver buffer. Pulsing without fentanyl was carried out with the citrate buffer, the fentanyl solution replacing the buffer 1 min after the last pulse.

Measurement of fentanyl quantities according to skin depth was performed by tape stripping and tangential slicing (14). The skin directly under the location of the donor compartment was excised, pinned flat and tape-stripped 10 times (Scotch Cristal, 602, Cergy Pontoise, France). The remaining underlying skin tissue was flattened on a glass slide laid over dry ice. Four to five biopsies (0.5 cm × 0.5 cm) were taken and mounted on a cryostat (2800 Frigocut, N Reichert-Jung). Ten 40 μm slices were cut at −22°C, parallel to the skin surface and corresponding slices from the different biopsies were combined. Radioactivity of each strip and of the slices was further measured by β counting (15).

The results were expressed as means ± the standard deviation of the means (n = 3 to 9).

Chemical and radiochemical stabilities of fentanyl under high-voltage pulses exposure were demonstrated and, measured radioactivity was shown to correspond to fentanyl (9).

Electroporation

The electroporation device used was an Easyject Plus® (Equibio, Seraing, Belgium) delivering exponentially-decaying capacitive discharge pulses. The pulse time (τ) is defined as the length of time between the beginning of the pulse (maximum voltage) and the time when the voltage reaches 37% of its initial value. It depends on the electrical circuit resistance, i.e., the shunt resistance of Easyject Plus® and the resistance of the diffusion chamber during the pulse ($R_{chamber}$) and, the capacity of the electroporation apparatus: τ = resistance × capacity. The around 600 ms pulses used in this study were generated with the highest resistance and capacity of the device (2310 Ω and 3000 μF). The energy of the electrical pulses (E) applied to the solutions and skin was calculated from the equation $E = N \cdot \tau \cdot (V_i^2 - V_f^2)/2 \cdot R_{chamber}$, where N is the number of the electrical pulses, V_i is the initial applied voltage and V_f is the final applied voltage (9). The pulse spacing was 1 min. Transdermal voltages which were approximately evaluated by the ratio of the skin resistance to the total chamber resistance were approximately 30% of applied voltages (4,5,9). Voltages are expressed as applied values and not transdermal values.

Statistical Analysis

The ratios of cumulative quantities detected in the receiver compartment to the skin area were compared by Student t-test ($p < 0.05$ or <0.01). The kinetics of drug permeation and the distribution profiles of fentanyl quantities in SC or viable skin were compared by a two way analysis of variance (Scheffé F-test, $p < 0.05$ or 0.01).

RESULTS

Mechanisms Involved in Transdermal Fentanyl Transport by Skin Electroporation

Fentanyl Transdermal Transport During Pulsing vs After Pulsing

As shown in the previous paper (9), skin electroporation allowed to increase and moreover to control the transdermal permeation of fentanyl *in vitro*. The cumulative quantities were shown to increase progressively with time and to appear after a lag time in the receiver compartment. Fentanyl was maintained in the donor compartment for the 6 h of the experiments. Therefore, fentanyl transport could occur essentially during pulsing with a progressive release thereafter from a drug reservoir created within the skin and/or could occur through electropermeabilized skin after pulsing ceased.

To test if some amount of fentanyl permeated the skin after pulsing and to evaluate the skin reservoir, the donor compartment was emptied of fentanyl after five (100 V − 675 ms) pulses. These electric conditions were selected as they were previously used in the study of the mechanisms involved in transdermal permeation of metoprolol by electroporation (5). In this case, if we observe no more increase in fentanyl transdermal permeation, it would mean that fentanyl crosses the skin only after pulsing. On the other hand, if we observe the same permeation, a fentanyl reservoir is created within the skin during pulsing, progressively releasing fentanyl afterwards. Finally, if we observe a decrease in fentanyl permeation, both mechanisms are involved. Fig. 1 shows that when fentanyl was present in the donor only during pulsing, fentanyl transport was significantly lower than when fentanyl was also present after pulsing (approximately two-fold lower, F-test $p < 0.01$). Therefore, a significant part of fentanyl was transported after pulsing indicating that pulsing altered skin structure, creating changes in skin permeability which persisted after pulsing. However, fentanyl permeation was still high indicating that electroporation induced an important skin reservoir.

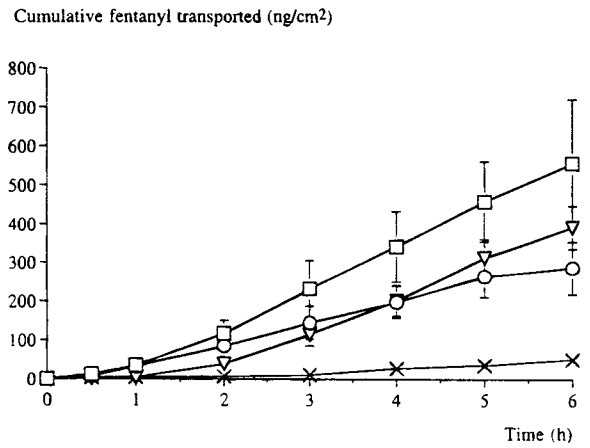

Fig. 1. Cumulative fentanyl transported (ng/cm^2) vs time (h) after passive diffusion (X) or, after electroporation (5 × (100 V − 675 ms)) as a function of the time at which fentanyl was present in the donor compartment: (□) during and after pulsing, (○) only during pulsing, (▽) only after pulsing. Fentanyl 40 μg/ml was in a citrate buffer pH 5 0.01 M.

To assess the lasting changes in skin permeability, the skin was not exposed to fentanyl during pulsing. Immediately after pulsing, fentanyl was placed in the donor compartment. In this case an elevated post-pulse transdermal flux could not originate from effect during pulsing but would instead be caused by electrically-induced changes in skin permeability. In this experiment (fig. 1), fentanyl permeation measured after pulsing was approximately 2-fold lower than when fentanyl was present during and after pulsing (F-test $p < 0.01$). However, compared to diffusion through untreated skin, diffusion of fentanyl through pulsed skin increased by an order of magnitude (fig. 1).

Approximately half of the cumulative amount of fentanyl observed at 6 h came from transport during pulsing and the other half came from transport after pulsing (fig. 1). However the amount transported after pulsing was the result of 6 h transdermal diffusion, while more than this amount was transported during only five minutes pulsing. This leads to the conclusion that the strongest effect of electroporation on fentanyl transport occurred during pulsing.

In order to check whether the proportion of the amounts transported during pulsing vs after pulsing could change with the electrical protocol, fentanyl transport was studied after different pulsing conditions with fentanyl present either during and after pulsing or, only present during pulsing (Table I). The cumulative quantity of fentanyl permeating the skin after 6 h increased with the energy of the applied pulses when fentanyl was present during and after pulsing as well as only during pulsing. Although electropermeabilization of the skin after pulsing increased with the energy, the transport during pulsing increased in almost the same proportion (Table I).

Electrophoresis and Diffusion in Fentanyl Transdermal Transport During Pulsing

The first experiments showed that fentanyl transport by skin electroporation involved a rapid elevated transport during pulsing and a slow passive transport after pulsing through electropermeabilized skin. The mechanisms of transport occuring during pulsing were then investigated. Different possible mechanisms could be involved: drug-electric field interaction (electrophoresis), convective flow (electroosmosis) and/or diffusion through skin highly permeabilized by electroporation.

Experiments were performed to check whether the electrophoretic movement was involved in fentanyl transport during pulsing. In a first experiment the effect of increasing competitive ions was assessed by increasing the concentration of the buffer ten-fold (0.1 M instead of 0.01 M). In a second experiment the electrode polarity was reversed, i.e. the cathode was placed in the donor compartment. Pulsing was carried out with five (100 V − 470 ms) pulses. Due to the increase in buffer concentration, the 100 V pulses were delivered in 470 ms instead of 675 ms, the duration of the pulses delivered with the buffer 0.01 M were consequently adjusted.

The cumulative quantities of fentanyl transported 6 h after the five (100 V − 470 ms) pulses did not decrease significantly when competitive ions were added in the donor or, when the electrode polarity was reversed (fig. 2a; t-test $p > 0.05$).

This experiment was perfomed with fentanyl present in the donor compartment during and after pulsing; since significant amounts of fentanyl crossed the skin after pulsing, the mechanisms involved during pulsing could not be apparent. To better evaluate the mechanisms involved during pulsing, the same experiment was performed with fentanyl present in the donor compartment only during pulse application. As shown in fig. 2b, competitive ions added in the donor compartment and the inversion of the electrode polarity decreased the cumulative fentanyl transported after 6 h (t-test, $p < 0.05$). When the buffer concentration was 0.1 M, 25% of the cumulative fentanyl amount observed at 6 h was transported during pulsing whereas 50% was transported at 0.01 M concentration.

These results clearly indicate that the role of the electrophoretic movement is important in fentanyl transport during pulsing. The amount of drug permeating the skin was still high when the cathode was in the donor showing that increased diffusion during and/or between pulses also represented an important contribution in fentanyl transport and that great partially reversible changes in skin permeability occurred during pulses (8).

Electroosmosis in Fentanyl Transdermal Transport During Pulsing

Because of the skin net negative charge, the convective water flow i.e. electroosmosis is in the anodal to cathodal direction (1,16–19).

To evaluate if electroosmosis was involved in transdermal drug transport during pulsing, fentanyl permeation was studied with fentanyl in an uncharged condition: the pH of fentanyl solution was fixed at pH 10 by a borate buffer (0.05 M). In this case, if fentanyl quantities permeating the skin are lower when the cathode instead of the anode is in the donor compartment, it could only be explained by electroosmosis.

As this pH is high and could be harmful for the skin, the fentanyl solution was in the donor just 6 minutes, the time necessary to apply pulses. The safety of the skin contact with the pH 10 solution was assessed by measuring skin permeability with tritiated water: the mean transdermal flux of tritiated water was not significantly increased after the contact with the pH 10 solution (2.1 ± 0.5 μl/cm^2 · h) compared to the contact

Table 1. Amount of Fentanyl Transported Through the Skin 6 h After the Application of Different Pulsing Conditions. Fentanyl Was Present in the Donor Compartment Either During and After Pulsing (1), or Only During Pulsing (2)

Pulsing Conditions	E	(1)	(2)	%
5 × (50 V − 765 ms)	18	301 ± 86	147 ± 24	49
5 × (100 V − 200 ms)	24	253 ± 51	83 ± 9	33
60 × (300 V − 1.6 ms)	26	248 ± 25	122 ± 20	42
5 × (100 V − 470 ms)	48	374 ± 61	215 ± 44	57
5 × (250 V − 120 ms)	67	485 ± 73	269 ± 40	56
5 × (100 V − 675 ms)	67	555 ± 163	296 ± 87	53

Note: Fentanyl (40 μg/ml) was introduced in a citrate buffer 0.01 M at pH 5. E (J) is the applied electrical energy of the pulses. (1) and (2): Cumulative fentanyl transported through the skin after 6 h (ng/cm^2) (± sdm) as a function of the time at which fentanyl was present in the donor compartment: (1) during and after pulsing, (2) only during pulsing. % = percentage of the amount of fentanyl transported during pulsing (2/1).

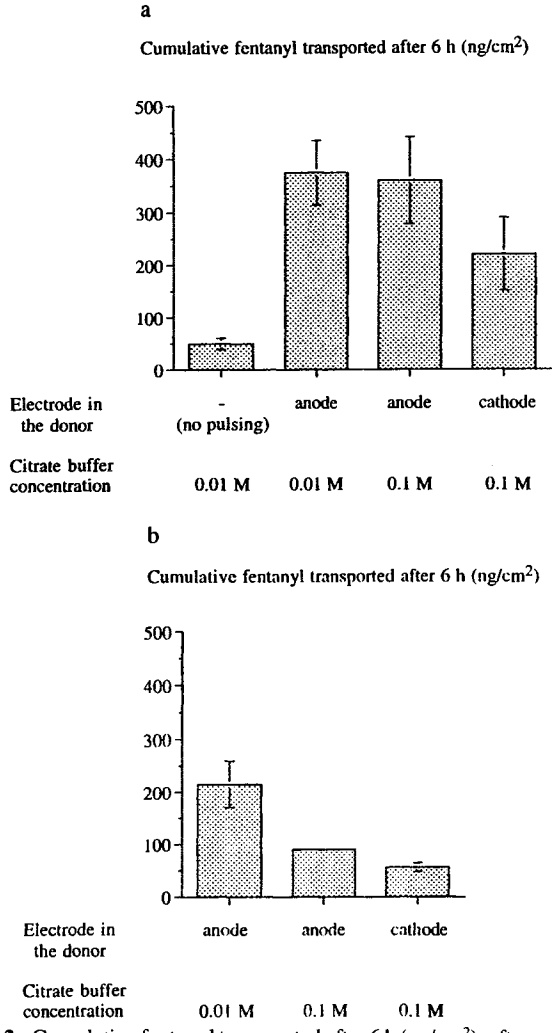

Fig. 2. Cumulative fentanyl transported after 6 h (ng/cm^2): after passive diffusion (**a**), or after electroporation (5 × (100 V − 470 ms)) (**a** and **b**) with the anode in the donor compartment and fentanyl (40 μg/ml) introduced in a citrate buffer pH 5 0.01 M or, 0.1 M or, with the cathode placed in the donor compartment and fentanyl (40 μg/ml) introduced in a citrate buffer pH 5 0.1 M. **a:** fentanyl was present in the donor compartment during and after pulsing. **b:** fentanyl was present in the donor compartment only during pulsing.

with a pH 7.4 saline solution (2.8 ± 0.2 μl/cm^2 · h) (t-test, $p > 0.05$).

Fentanyl permeation was studied after five (100 V − 675 ms) pulses with either the anode or the cathode in the donor compartment and, after the simple contact with the pH 10 solution. As expected the diffusion of uncharged fentanyl was high. In comparison with diffusion, skin electroporation enhanced fentanyl permeation only 2-fold showing that the transport induced by pulsing was not important (fig. 3; F-test, $p < 0.01$). No significant difference in fentanyl permeation was observed when the anode or the cathode was placed in the donor (F-test, $p > 0.05$). Similar results with neutral molecules (tritiated water and mannitol) tend to indicate that electroosmosis is not involved in the mechanisms by which electroporation enhances drug permeation (20).

Measurement of Fentanyl Quantities in the Skin

Measurement of fentanyl quantities in the skin was performed after skin electroporation and compared to passive diffusion. The measurement was carried out in the SC and in the epidermis/dermis separately and according to their depth (15).

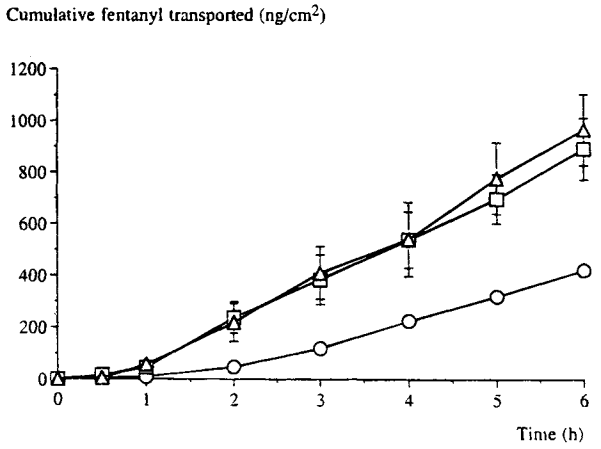

Fig. 3. Cumulative fentanyl transported (ng/cm^2) vs time (h) after passive diffusion (○) or, after electroporation (5 × (100 V − 675 ms)) with the anode (△) or the cathode placed in the donor compartment (□). Fentanyl 40 μg/ml was in a borate buffer pH 10 (0.05 M) and present in the donor compartment only during pulsing.

Five high voltage pulses of (250 V − 70 ms) and five lower longer pulses of (50 V − 1 s) were applied to the skin, fentanyl was present in the donor compartment during and after pulsing. Either immediately or 1 h after pulsing, the skin was tape stripped and tangentially sliced. Both pulsing conditions provided the same cumulative quantity of fentanyl in the receiver after 6 h (350 ng/cm^2). These electroporation conditions were chosen to check if different electrical conditions giving an identical permeation profile would also give a similar distribution profile of fentanyl quantities in the skin.

Compared to passive diffusion, both skin electroporation conditions did not increase fentanyl quantities detected in the SC either when the stripping was done immediately after pulsing or when the stripping was done after 1 h (fig. 4a, F-test $p > 0.05$). In all cases, identical distribution profiles were observed with the upper layers closer to the fentanyl solution containing greater quantities.

Immediately after pulsing, fentanyl penetration is enhanced by an order of magnitude in the epidermis and dermis as compared to diffusion (fig 4b; F-test, $p < 0.01$). The enhancement was homogeneous through all slices of the skin. The 250 V and 50 V conditions were equivalent in their promoting effect

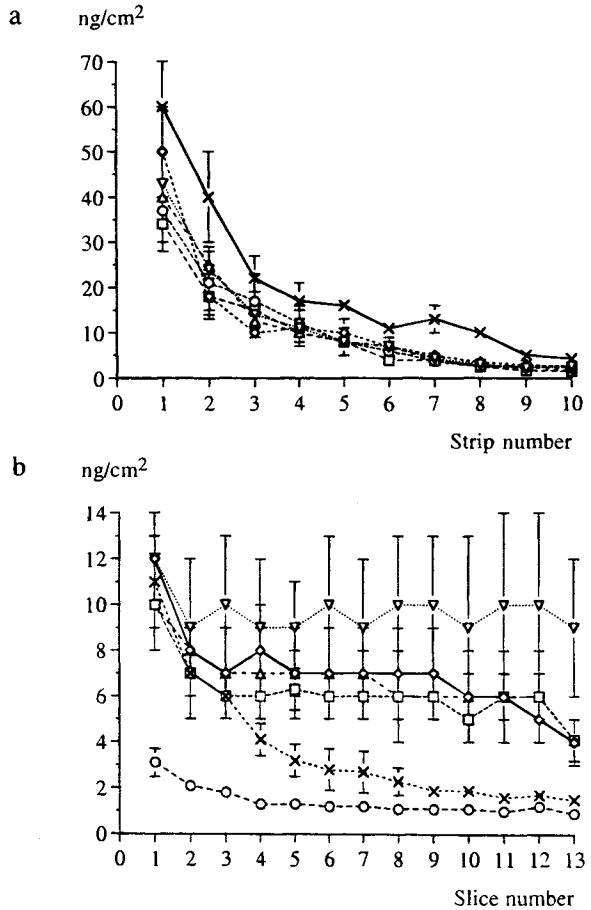

Fig. 4. Quantity of fentanyl (ng/cm^2) in the skin directly or 1 h after electroporation with (5 × (50 V − 1 s)) pulses (△ or ▽) or with (5 × (250 V − 70 ms)) (□ or ◇) or, after the corresponding passive diffusion (○ or X). Fentanyl 40 μg/ml was in a citrate buffer pH 5 0.01 M. **a:** as a function of stratum corneum strip. **b:** as a function of epidermis and dermis depth (slices 40 μm thick).

(F-test $p > 0.05$). One hour after pulsing, the difference between skin electroporation and diffusion in fentanyl accumulated quantities remained significant (F-test, $p < 0.01$). After 1 h diffusion through untreated skin, fentanyl quantities increased in the upper slices approaching quantities obtained by skin electroporation.

DISCUSSION

Mechanisms Involved in Transdermal Molecular Transport by Skin Electroporation

This study points out that transport during and after pulsing are both important in transdermal delivery of fentanyl by skin electroporation. Mechanisms involved during pulsing were shown to be electrophoresis and diffusion through highly permeabilized skin. An important skin reservoir created by electroporation progressively released fentanyl for at least several hours. No electroosmosis emerged. The increase in post-pulse permeation was due to enhanced diffusion through durably altered skin. It could however also be explained by fentanyl lipophilicity leading to an easy diffusion through the altered lipophilic skin membrane.

Previously we studied some mechanisms behind improved transdermal delivery by electroporation of metoprolol, a low molecular weight hydrophilic drug (267 Da, $\log D_{oct/water\ at\ pH\ 7.4} = -0.23$) (5). Reversible increased skin permeability, electrophoretic movement of the drug during pulse application and drug release from the skin reservoir formed by electroporation were involved. Only a slight proportion of the transport occured after pulsing. Therefore, the mechanisms were found to be similar to what happened with fentanyl. Nevertheless while inversion of the electrodes polarity resulted in a decrease of an order of magnitude in metoprolol transdermal permeation, it resulted only in a smaller decrease in fentanyl transdermal permeation (fig. 2). This suggested that in contrast with metoprolol, the contribution of increased skin permeability in fentanyl transdermal transport by electroporation is more important. Lipophilicity of fentanyl is higher than metoprolol and hence its diffusion through the electroporated skin could be easier. In addition, ions were shown to be in competition with the drug in the transport and the physicochemical properties of the donor solution for both studies were quite different (5; fig. 2). Fentanyl (10^{-4} M) was 100-fold less concentrated than its citrate buffer (0.01 M) meaning that fentanyl carried 0.3% of the total ions electric charges of the solution, while metoprolol (0.03 M) was 3-fold more concentrated than its phtalate buffer (0.01 M) meaning that metoprolol carried 37% of the total charges. Therefore the electrophoretic movement affected mainly the buffer ions in the fentanyl study whereas, it affected the drug to a greater extent in the metoprolol study.

Pliquett et al (8) comparing calcein (−4 charged polar molecule, 623 Da) and sulforhodamine (−1 charged polar molecule, 607 Da) transport by electroporation have also shown that the contribution of electrophoresis was important for calcein while diffusion through dramatically and reversibly altered skin dominated for sulforhodamine. Prausnitz et al (12) demonstrated that high-voltage pulses increased the transport of heparin, a highly-charged macromolecule (charge = −76, average MW of 12 kDa) at therapeutic rates across human epidermis *in vitro*, emphasizing again how important physicochemical

properties of the transported molecule are in drug transport by skin electroporation.

These data demonstrate that the contribution of the different mechanisms involved in molecular transport by electroporation depends on the physicochemical parameters of the transported molecule i.e. octanol/water partition coefficient, electric charge, size as well as, the solution i.e. drug concentration, vehicle and background electrolyte (5,7,8,12). Hence the consideration of formulation variables will be essential for the development and optimization of skin electroporation device.

Comparison of Electroporation and Iontophoresis

Iontophoresis may be described as a process in which drug molecules experience electrically assisted movement into and through the skin following an electrical potential difference (0.1 to 5 V across the skin) and that no new routes of passage are created. Mechanisms involved in iontophoresis transport were shown to be mainly electrophoresis and electroosmosis, increase in skin permeability being a secondary process (16–19).

While iontophoresis is assumed to act mainly on the drug, skin electroporation is assumed to act mainly on the skin. As reported previously electrophoresis alone is unable to explain the enhancement of fentanyl permeation by high-voltage pulses indicating that skin structural changes must occur (9). The experiences reported here provide additionnal proofs of skin alterations. The increase in skin permeability during pulsing is indicated by high fentanyl flux during pulsing with the inverted polarity at pH 5 and, at pH 10, and the persistence of the increased permeabilization is indicated by the lasting increased diffusion after pulsing (fig. 1, 2).

An other striking difference between iontophoresis and skin electroporation is electroosmosis. While electroosmosis was demonstrated to govern the iontophoretic permeation of neutral and even some charged compounds, the convective flow did not seem to be involved in skin electroporation (fig. 3; 16–19).

Since the electrophoretic movement is involved in both methods, equal attention must be given to physicochemical properties of transported molecules as well as the solution. The introduction of competitive ions in the medium decreased drug transport by iontophoresis (17). The iontophoretic enhancement factor of fentanyl transdermal fluxes was higher after introduction of the drug into an acidic medium as a result of the greater fraction of ionized drug compared to the introduction into a neutral solution (18). It was observed that simple skin contact with the pH 10 solution allowed fentanyl transdermal permeation to the same extend as with five (100 V − 675 ms) pulses at pH 5 (fig. 1 and 3). But one should consider that the drug concentration used in this study is not adequate to obtain significant rate *in vivo* and that higher concentration is not feasible at pH 10 because of fentanyl solubility (21).

Measurement of Fentanyl Quantities in the Skin

In SC, the study did not show any significant difference in the distribution of fentanyl quantities after diffusion or electroporation (fig 4a). This could easily be explained by the fact that due to its lipophilicity, fentanyl partitioned easily in SC. For a very lipid soluble drug, the affinity for SC may be so high that the clearance from the horny layer replaces diffusion through SC as the rate-limiting step (15).

Electroporation loaded the epidermis/dermis with fentanyl immediately when pulsing was applied (fig 4b). Moreover one hour after pulsing, the fentanyl quantities did not decrease suggesting that fentanyl entrapped in an aqueous environment did not diffuse easily afterwards or, that the release from the SC balanced the release in the receiver. Fentanyl quantities measured in viable skin (slice 40 μm thick) are dramatically lower than in SC (strip 0.8–0.9 μm thick).

The *in vitro* model of this study was full-thickness hairless rat skin. Since capillaries exist near the dermal/epidermal junction, drugs can enter the systemic circulation without crossing the whole dermis (1). Thus, the dermis of full-thickness skin is known to induce *in vitro* a prolongation of the lag time for detecting the drug in the receiver compartment and may keep the drug trapped: the cell mass of epidermis/dermis beneath the SC contributes to an aqueous resistance to diffusion. These facts and *in vivo* data (21) suggest that the fentanyl reservoir observed in the experiments of the present study with the long lag time to detect fentanyl in the receiver should be considered carefully and seems to be mostly the consequence of the skin model. Moreover Pliquett et al (8) who used human epidermis showed in contrast that high-voltage pulsing during 1 h caused large and very rapid increase in molecular flux: the passage of molecules through the skin was shown to occur mainly during pulsing, either a few minutes lag time or none was observed and after pulsing ceased only a small fraction of molecular transport occurred.

CONCLUSION

Skin electroporation has been shown to significantly increase transdermal permeation of small-size drugs as well as considerably larger molecules. However mechanisms involved in enhanced transport have generally been incompletely investigated, although their comprehension is essential for the optimization of drug transdermal delivery.

The transdermal delivery of fentanyl by electroporation was studied in our previous report concerning the influence of the electrical factors (9). The present study explored the mechanistic aspects of fentanyl transport by electroporation. Significant transport occured both during and after pulsing. The rapid elevated transport during pulsing involved electrophoresis and diffusion through highly electropermeabilized skin. Electroosmosis was shown not to be a mechanism of transport. Significant slow transport occurring after pulsing was due to easier diffusion through durably electropermeabilized skin. Finally, measurements of fentanyl quantities in the skin demonstrated that electroporation rapidly loaded the viable part of the skin with fentanyl and hence rapidly overcame skin barrier.

The mechanisms involved in fentanyl transport by electroporation reported in this study concur with what has been previously reported for other molecules. However, the study points out that the contribution of the different mechanisms involved in molecular transport by electroporation strongly depends on the physicochemical parameters of the transported molecule as well as the solution, emphasizing the fact that formulation in skin electroporation device will require careful consideration.

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