SUPPLEMENTARY INFORMATION

Further analysis of data presented in main body text showed flux of BUP is dramatically influenced by choice of receptor solution

Fig S1 shows effect of receptor solution on flux, all other experimental parameters were identical.

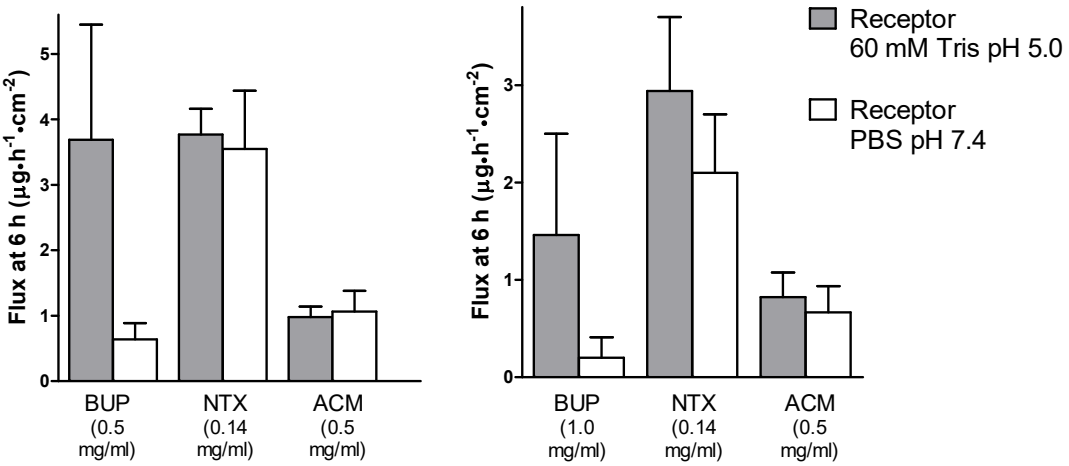

Fig S1: Iontophoretic J_{BUP}, J_{NTX} and J_{ACM} as a function of receptor solution; receptor solution was either 60 mM Tris pH 5.0 or PBS pH 7.4 (combined data from Part A). Background electrolyte of donor was 60 mM Tris pH 5.0. Values are mean + standard deviation. n = 4-9

Choice of receptor solution had no significant effect on J_{NTX}, however, in contrast, J_{BUP} was significantly (~6-fold) lower using PBS pH 7.4 as a receptor solution compared to using Tris pH 5.0. The low J_{BUP} with PBS could not be explained by absence of sink conditions; BUP concentrations in the PBS receptor samples were ≥ 10-fold lower than BUP solubility in PBS. Nor could the reduction of J_{BUP} observed be explained by inhibition of EO flow (no statistical difference in J_{ACM}). Furthermore, the amount of BUP in the stratum corneum (or tissue where assayed) was not significantly different for the two receptor solutions.

Tests were therefore performed using receptor solutions of differing ionic strength, pH, buffer type and/or concentration of chloride.

Further investigation showed receptor solution composition has a large, and *contrasting*, effect on iontophoretic flux of BUP and NTX, primarily influenced by pH

To investigate the observed sensitivity of J_{BUP} to receptor solution, experiments were carried out using three further receptor solutions; all other experimental parameters were identical. Figure S2 shows the results.

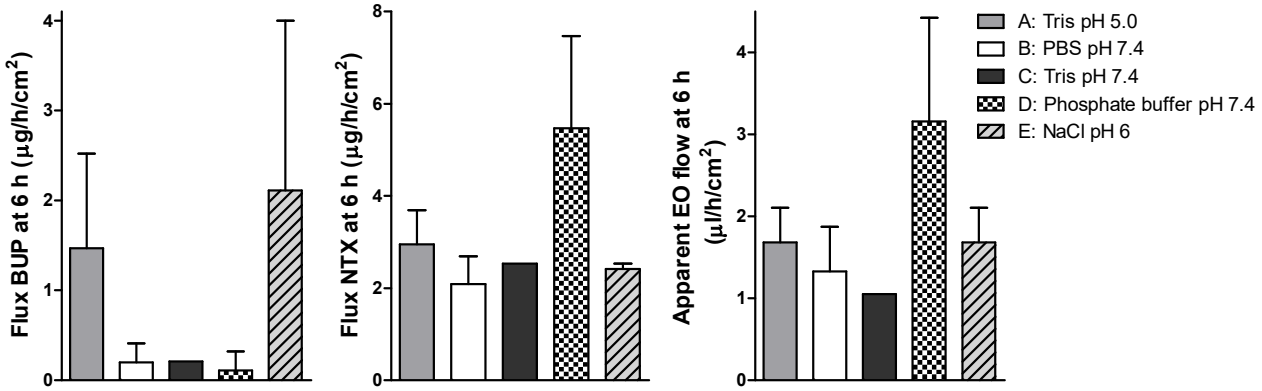

Figure S2: J_{BUP} and J_{NTX}, and apparent EO flow (mean + standard deviation) for different receptor solutions. The donor solution was 0.14 mg/ml NTX, 1.0 mg/ml BUP and 0.5 mg/ml ACM, in 60mM Tris pH 5.0 (n = 6, 9, 2, 5, and 4). Receptor solutions were **A**: Tris pH 5 (60 mM) **B**: Phosphate buffered saline pH 7.4 (153 mM) **C**: Tris buffer pH 7.4 (16 mM Tris HCl and 126 mM Tris base) **D**: Phosphate buffer pH 7.4 (15 mM) or **E**: NaCl pH 6 (138 mM NaCl).

Choice of receptor solution had a clear effect on J_{BUP}, J_{NTX}, and on EO flow. Interestingly, contrasting effects were observed for BUP and NTX (see the reversal of effect on flux for receptor solutions D and E). Results showed that BUP's sensitivity to receptor solution could not be attributed to buffer type (see B versus C) nor to increased counter-ion competition (see B versus D). There was however, a possible effect of pH (see B, C, D versus A and E). This observation is compatible with the proposed theory of slow BUP partitioning from skin into PBS pH 7.4 due to BUP's pH-related aqueous solubility.

These data were generated to investigate the behaviour of BUP, but a comment on the NTX fluxes is offered. The higher flux of NTX into receptor solution D could be due to either:

1. The absence of chloride ions in receptor solution D. In the absence of chloride, phosphate is the major anion in the receptor fluid, but as it is larger and less mobile than chloride, it is less efficient at competing with NTX to carry available charge (Phipps & Gyory 1992). It was not the aim of this section to investigate competition with counter-ions, but this data does highlight how the efficiency of iontophoretic delivery of cations will always be limited by the presence of ubiquitous endogenous chloride in the subdermal compartment *in vivo* (Burnette & Ongpipattanakul 1987). Furthermore, it is expected that less mobile ions (such as BUP), respond proportionally less than more mobile ions (such as NTX) to changes in the composition of counter-ions in the receptor solution (Mudry et al 2006).

2. The low ionic strength (15 mM) of receptor solution D. Ionic strength is inversely related to EO (Tamada & Comyns 2005). The corresponding increase in J_{ACM} is consistent with the increased flux of NTX being driven by EO.

Results indicated that the property of the receptor solution which most influenced J_{BUP} was pH.

Overall, this small data set implies that effect of receptor solution composition on flux can be large, and is permeant-specific.

Receptor solution composition has a large, and *contrasting*, effect on passive release of BUP and NTX out of the skin following a period of iontophoresis

It was hypothesised that after being driven into the skin by iontophoresis, the movement of BUP from the tissue into the receptor was largely due to passive diffusion down a concentration gradient (Sage & Riviere 1992; Singh *et al*. 1994), and that the rate was strongly influenced by the solubility of BUP in the receptor solution. Aqueous solubility of BUP drops from 12 to 0.045 mg/ml between pH 5 and pH 7.4 (Robson 1988), thus partitioning of BUP out of the skin into the receptor might occur more slowly at the higher pH. To test whether J_{BUP} into PBS pH 7.4 was being limited by the drug's low solubility at this pH, an experiment was carried out in which post-iontophoresis passive release rates were measured.

Method: Between 0 and 6 hours, the donor solution was 1.0 mg/ml BUP, 0.14 mg/ml NTX, and 0.5 mg/ml ACM in 60 mM Tris pH 5.0, and the receptor solution was PBS (phosphate-buffered saline pH 7.4). Current was terminated after 6 hours. The donor solution was replaced with PBS only, and the receptor solution was replaced with 60 mM Tris buffer pH 4.0. Passive release of NTX and BUP from the skin into the receptor chamber was then measured during the following 18 hours.

BUP and NTX were delivered by iontophoresis for 6 hours, with a receptor solution of PBS pH 7.4. At 6 hours, the current was terminated, and the receptor solution was replaced with one in which BUP is freely soluble (Tris pH 4 was chosen for this purpose; the aqueous solubility of BUP at pH 4 is ~19 mg/ml; Robson 1988). All drug was removed from the donor compartment, and passive release of drug from the skin into the receptor compartment was measured for a further 18 hours. If J_{BUP} was greater into a receptor solution of pH 4 (no current) than into a receptor solution of PBS pH 7.4 (with current) this would indicate that during iontophoresis with PBS as a receptor solution, J_{BUP} is limited by partitioning from the viable skin into the aqueous receptor solution. Fig. S3 shows the results.

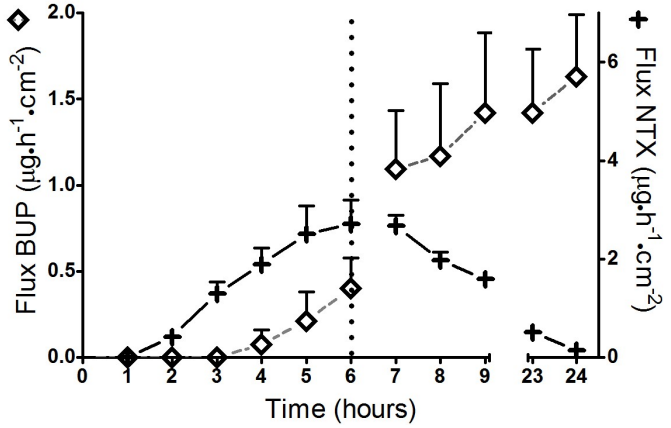

Fig. S3: Iontophoretic fluxes (0-6 hours), and post-iontophoretic release of BUP and NTX from the skin (6-24 hours) (mean + standard deviation, n = 6). Donor solutions contained 0.14 mg/ml NTX, 1.0 mg/ml BUP and 0.5 mg/ml ACM in a background electrolyte of 60mM Tris pH 5.0. The vertical dotted line indicates termination of current, removal of donor solution, and switching the receptor solution from PBS pH 7.4 to Tris pH 4.0.

Despite termination of current and removal of all drug from the donor J_{BUP} (but not J_{NTX}) *increased* ($p < 0.05$) when, at 6 hours, receptor solution was changed from PBS pH 7.4 to Tris pH 4. This observation supports the hypothesis that when PBS pH 7.4 was used as the

receptor solution, distribution of BUP from the skin into the receptor during iontophoresis was rate-limiting. This provides a credible explanation for the significant effect of choice of receptor solution on J_{BUP} but not on J_{NTX} (Fig. S1); NTX has much higher aqueous solubility than BUP. It is possible, therefore, that slow partitioning of BUP from the skin tissue into a receptor solution of PBS pH 7.4 might result in underestimation of *in vivo* fluxes. This might be addressed *in vitro* by considering drug reaching the dermis as systemically 'delivered', however, in this work, the dermis and viable epidermis were not separated before extraction for assay, so no such calculation was made.

Method detail: Preparation and conditioning of electrodes

Silver wire (0.5mm ø Sigma) was cut into lengths of ~5cm. AgCl (Sigma) was deposited on the wire by repeatedly dipping the wire into the molten AgCl. These AgCl electrodes were ready to use immediately as cathodes. In order to use for anodes, the AgCl electrodes were conditioned, by running as a cathode against platinum wire (0.2mm ø Sigma), overnight at 0.2 mA with 50 mM NaCl as an electrolyte.

References

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