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Effect of penetration enhancers on the permeation of the thyrotropin releasing hormone analogue pGlu-3-methyl-His-Pro amide through human epidermis

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Abstract

The effect of the enhancers, cineole and ethanol, on the transdermal penetration of the tripeptide, pGlu-3-methyl-His[2]-Pro amide (M-TRH), across human epidermal membrane was studied by flow-through diffusion chambers. The aim of the study was to assess whether the biologically active analogue M-TRH displays similar transdermal penetration properties as those demonstrated earlier for the parental peptide, thyrotropin-releasing hormone (TRH) (Magnusson et al., 1997a Int. J. Pharm. 157, 113–121). Steady-state fluxes with a donor solution of phosphate-buffered saline (PBS) were 0.34 ± 0.01 μg/cm^{2}h for M-TRH and 0.27 ± 0.01 μg/cm^{2}h for TRH. Measured over 30 h the total amount penetrated was 8.6 ± 1.0 and 7.8 ± 1.7 μg/cm^2, respectively. In the presence of 50% ethanol, the flux of the peptides increased approximately 3-fold. A donor solution of 3% cineole, in combination with 47% ethanol, increased the penetration of M-TRH to 1.60 ± 0.02 μg/cm^{2}h, compared to 0.92 ± 0.03 μg/cm^{2}h for TRH, as reported previously. The corresponding total amount penetrated over 30 h was 41.5 ± 4.9 and 24.9 ± 1.7 μg/cm^2, respectively. Our data suggests that enhancers added together with the penetrant can theoretically induce changes in the permeability of the stratum corneum sufficient to promote the transdermal absorption of therapeutically relevant amounts of these peptides. This demonstrates the possibility to deliver classes of compounds that have been viewed as not suitable for transdermal administration.

Keywords: Diffusion chamber; Penetration enhancers; Peptides; Transdermal delivery

1. Introduction

Thyrotropin-releasing hormone (TRH) is a neuropeptide with a primary structure, L-pyroglutamyl-L-histidyl-L-proline amide (pGlu-His-Pro-

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NH_2), that stimulates the release of thyroid-stimulating hormone (TSH) and prolactin from the pituitary. The potential therapeutic applications that have attracted the most attention are not based on its endocrine properties, but on its broad spectrum of stimulatory actions within the central nervous system (CNS). These CNS-mediated effects provide the rationale for the use of TRH in the treatment of brain and spinal cord injury and certain CNS disorders, including Alzheimer's disease and motor neuron disease (MND). The beneficial effects of TRH on CNS-disorders and trauma appear to be partly due to its ability to potentiate other neurotransmitter systems and to reverse or attenuate certain actions of secondary injury factors that occur as a result of CNS-trauma (Horita et al., 1986). However, the exact mechanism by which TRH improves these conditions is still not fully understood.

There are many reports on the effects of i.v. administration of synthetic TRH on TSH, prolactin and thyroid hormone release in man, and plasma TRH concentrations obtained (Mitsuma and Nogimori, 1984; Schurr et al., 1985). Surprisingly little interest has been shown in alternative routes of administration of TRH. Transdermal delivery is an attractive option. It avoids gastrointestinal degradation and the hepatic first-pass effect, lends itself to controlled, sustained delivery, and encourages patient compliance since a transdermal delivery system would be easy to apply and remove. The basic prerequisite of transdermal drug delivery is the ability of the drug to penetrate the outermost layer of the skin, the stratum corneum. This is comprised of keratin-rich cells embedded in multiple lipid bilayers and is an excellent barrier to the transport of large molecules and charged compounds. Therapeutically effective rates of drug delivery are therefore difficult to achieve for these substances without some form of facilitation, such as chemical enhancement. Terpene compounds isolated from plant volatile oils are an example of a group of such enhancers. They act by partition into, and interaction with, the stratum corneum constituents to ideally induce a reversible increase in skin permeability (Williams and Barry, 1992).

It has been known for some time that TRH is degraded by enzymes at various sites in the body. The presence of enzymes at these sites may reflect, not only the ability to inactivate TRH, but also other functions such as regulating tissue content, duration of action and degradation into smaller, biologically active fragments. In an attempt to overcome the susceptibility of TRH to enzymatic degradation, various analogues have been synthesized. These have tended to incorporate modifications that provide stability to degradation by the pyroglutamyl aminopeptidases (PAP:s) and/or prolyl oligopeptidase (Metcalf, 1982; Hichens, 1983). Analogues that are stable to PAP activity have been shown to have enhanced CNS activity. The TRH analogue, pGlu-3-methyl-His^2-Pro amide (M-TRH), is a potent analogue and stimulates the release of TSH from the pituitary seven to eight times that of the parental tripeptide (Sowers et al., 1976). This analogue has certain advantages, such as enhanced binding to pituitary TRH receptors (Horita et al., 1986) and an increased specificity to the endocrine actions of TRH (Ward et al., 1987).

In an earlier study we reported the transdermal penetration of TRH through human epidermal membranes (Magnusson et al., 1997a). That study showed that a therapeutic dose of TRH could theoretically be delivered by the use of penetration enhancers. The purpose of the present study was to investigate the penetration of the analogue M-TRH through human epidermis using cineole and ethanol as penetration enhancers and to compare the result with that of the parental peptide. The chemical structures of the individual tripeptides and the enhancer are illustrated in Fig. 1.

2. Materials and methods

2.1. Materials

Unlabeled M-TRH (pGlu-3-methyl-His^2-Pro amide, lot. no. 73H06221) and TRH (pGlu-His-Pro amide acetate, lot. no. 46H5845) were supplied by Sigma (St. Louis, MO, USA). M-TRH [(L-histidyl-4-^{3}H(N), L-prolyl-3,4-^{3}H(N))-] and TRH [(L-proline 3,4-^{3}H(N), histidyl-3-^{3}H(N))-]

with a specific activity of 37 MBq/ml, were supplied by NEN Research Products (Dreichen, Germany). The terpene, cineole (99%), was obtained from Sigma (St. Louis, MO, USA). The receptor fluid used was phosphate-buffered saline (PBS) solution, pH 7.4.

2.2. Diffusion cells

The permeation studies used an automated diffusion system equipped with miniature diffusion cells containing flow-through receptor compartments. The details of the system have been previously described by Magnusson et al. (Magnusson et al., 1997a,b). In short, it consists of stainless steel blocks, each containing four diffusion cells, maintained at 32.0 ± 0.1°C and placed directly above a Fractomin® autosampler. Each cell has a nominal diffusion area of 0.50 cm^2. Sink conditions in the receptor compartment were achieved by a flow of degassed PBS solution. The composition of the donor solution was maintained by a supply of fresh solution through an intermittently running peristaltic pump with a net flow of 20 μl/h. The amount of donor solution was kept at 120–150 μl by a suction tube placed at the appropriate level in the compartment. Teflon plugs covered the donor compartments to prevent evaporation. All parts of the system in contact with donor or receptor solutions were made of stainless steel, teflon or polyethene. Two blocks giving a total of eight cells were used for each run.

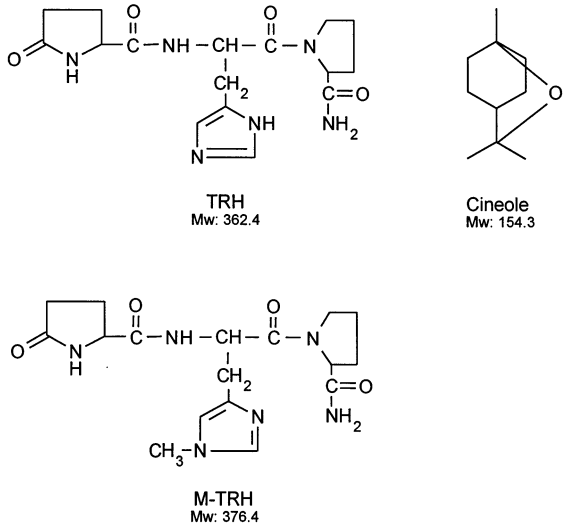

Fig. 1. The structural formulae of the tripeptides and the terpene used as a penetration enhancer.

2.3. Epidermal membranes

Human breast skin was obtained from plastic surgery, trimmed of subcutaneous fat and stored at -70°C. Epidermal membranes were prepared by immersing 13 mm disks of full thickness skin in water at 60°C for 90 s. The epidermal membranes could then be gently peeled off from the underlying dermis (Kligman and Christophers, 1963). As the membranes from hairy skin samples tended to tear during preparation, they were rejected. Skin rich in appendages (≥ 6 appendages per membrane) were discarded to avoid excessive transappendageal penetration. The epidermal membranes were hydrated for 18 h in PBS at 4°C before mounting in the diffusion cell. All the epidermal membranes in the present study were prepared from the same skin source.

2.4. Diffusion experiments

Prior to any test, the integrity of the stratum corneum barrier was verified for each epidermal disk by the determination of water permeability. A 100 μl volume of [^{3}H]-H_2O solution (925 kBq/ml) was applied to the donor compartments. Receptor fluid with a flow through rate of 4.0 ml/h was sampled in 15-min intervals for a minimum of 1.5 h. A sample of 10 ml Ready Gel™ cocktail scintillation fluid was added to each sample and [^{3}H]-H_2O was determined by liquid scintillation counting (Beckman LS 5000 CE). Membranes with a water permeability of < 5.0 μl/cm^2h were accepted for the diffusion experiment. The [^{3}H]-H_2O was removed from the membrane with PBS solution for a minimum of 3 h, while continuously replacing the donor solution. This step reduced the residual [^{3}H]-activity to background levels.

In all tests, the concentration in the donor solution of M-TRH was 5.0 mg/ml and the [^{3}H]-activity 370 kBq/ml. Penetration of M-TRH was measured using the following three compositions:

(1) PBS for the determination of base-line levels of M-TRH flux; (2) 50% (w/w) PBS and 50% (w/w) ethanol to study the effect of ethanol on the penetration of M-TRH; and (3) 3% (w/w) cineole, 47% (w/w) ethanol and 50% (w/w) PBS to study the effect of the terpene on the M-TRH flux. To study the early phase of enhancer-induced changes in permeability, all components of the donor solutions were mixed before they were added to the donor compartment. The 3% level of cineole was chosen on the basis that it is the maximum concentration allowing for homogenous solution with the levels of PBS and ethanol. At the start of the experiment, 100 μl of the respective solution was applied to the donor compartment. The composition was maintained by the continuous renewal of the test solutions as described earlier. Perfusion fluid, with a flow of 1 ml/h, was sampled with an interval of 1 h over a 30 h period. The amount of M-TRH was assayed by liquid scintillation technique. The pseudo-steady state permeability coefficients, K_p (cm/h), were calculated from the steady-state flux, J (μg/cm^{2}h), and the donor concentration, C (μg/cm^3), using the relationship: $K_p = J/C$. The effect of the enhancers were expressed as enhancement factors (EF), which is the ratio of the drug permeability coefficient in the presence of enhancer to the permeation of the drug with PBS only.

2.5. Scintillation technique vs radio-immunoassay

To compare with previously published results (Magnusson et al., 1997a), penetration of TRH with a donor solution of PBS were performed as described above for M-TRH. This control was necessary since the epidermal membranes in the current study were donated from a different patient than that used in the previous TRH study. In addition, if degradation occurs, the liquid scintillation technique can provide false data by measuring labeled metabolites in addition to the intact peptide. To evaluate this, the TRH samples were collected and analyzed by both liquid scintillation technique as described above and by the radio-immunoassay method (RIA) used previously (Magnusson et al., 1997a). The commercial RIA (Euro-Diagnostica, Apeldoorn, The Netherlands) utilizes a polyclonal antibody raised in rabbit against TRH. It was performed according to the manufacturers directions with the exception that no extraction with ethanol was performed before the sample was analyzed. This has been shown not to be necessary (information by the supplier). As the RIA uses [^{125}I]-TRH, no interference from the radiolabelled [^{3}H]-TRH disturbed the assay. The assay has a detection limit of 5 pg/ml, corresponding to a flux of 10 pg/cm^{2}h under the present experimental conditions, and an interassay variation of less than 10%. As no RIA was available for M-TRH, this check had to be confined to the parental peptide TRH.

2.6. Statistics

The results were statistically analysed by Student's t-test (two-tailed) for unpaired observations. Statistical comparisons were performed between the M-TRH values in presence of ethanol and/or terpene vs the control penetration of the analogue. A difference was considered statistically significant if $p \leq 0.05$. Values are reported as mean $\pm$ SEM.

3. Results

All the epidermal membranes in the present study were prepared from the same skin source. The water penetration between the skin sources was compared, since the previous study by Magnusson et al. (1997a) was performed on a different individual. There was no statistically significant difference of water permeability between the epidermal membranes of the donors in this experiment compared to the previous study. The values were $3.0 \pm 0.1 \times 10^{-3}$ ($n = 16$) and $2.7 \pm 0.1 \times 10^{-3}$ ($n = 15$) cm/h, respectively.

In earlier penetration studies by Magnusson et al. (1997a), RIA was used to detect TRH. Since no RIA was available for M-TRH, the comparison between liquid scintillation technique and RIA had to be confined to the parental peptide TRH. As seen in Fig. 2, there was no statistically significant difference in TRH permeability when measured by liquid scintillation technique or RIA.

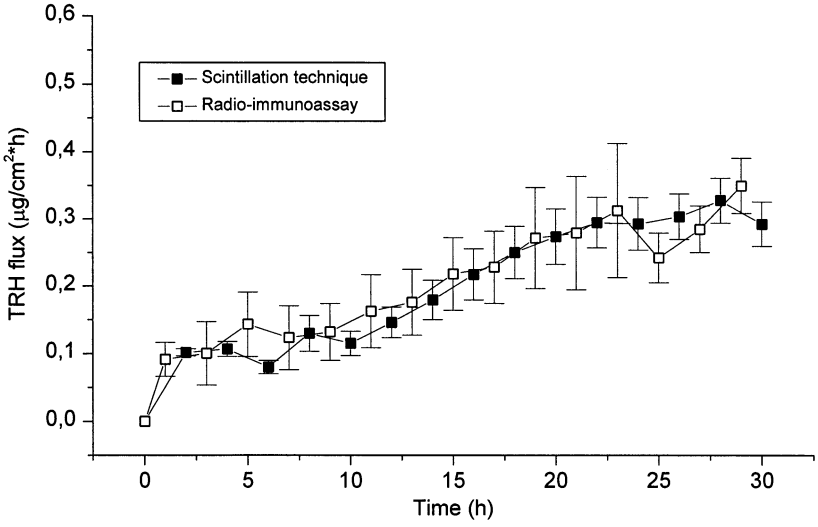

Fig. 2. Comparison of the TRH permeability through epidermal membranes using liquid scintillation technique and radio-immunoassay. Values are mean ± SEM ($n = 4$).

The mean values for TRH penetration under control conditions, analyzed by liquid scintillation technique and RIA between 14 and 30 h, were 0.27 ± 0.01 and 0.29 ± 0.01 μg/cm^{2}h, respectively (Fig. 2). The values of TRH penetration from a PBS donor solution obtained in this study are in agreement with data previously reported by Magnusson et al. (1997a).

Excised human skin was found permeable to the analogue of thyrotropin-releasing hormone as has been shown earlier for the parental tripeptide. Fig. 3a shows the penetration of M-TRH in the presence of PBS, PBS/ethanol and PBS/ethanol/cineole. For comparison, the corresponding results for TRH reported earlier in part by Magnusson et al. (1997a) are given in Fig. 3b. The mean permeability value of M-TRH under control conditions over 20–30 h was 0.34 ± 0.01 μg/cm^{2}h. This corresponds to a permeability coefficient of 6.8×10^{-5} cm/h (Table 1). The presence of ethanol increases the flux of M-TRH significantly when compared to the control conditions at 16 h and all subsequent observations ($p \leq 0.05$ Student's t-test). The mean value during the period 20–30 h was 1.01 ± 0.03 μg/cm^{2}h (Fig. 3a). The presence of 3% cineole and 47% ethanol increased the flux of M-TRH to 1.60 ± 0.02 μg/cm^{2}h over the period 20–30 h. Expressed as permeability coefficients, the penetration of M-TRH with ethanol and M-TRH in combination with ethanol/cineole, were 20.2 and 32.0×10^{-5} cm/h, respectively (Table 1). At 6 h, the flux of M-TRH in the presence of ethanol/cineole and ethanol alone were 1.38 ± 0.38 and 0.26 ± 0.03 μg/cm^{2}h, respectively. This early difference may be due to cineole being a more effective penetration enhancer than ethanol by its rapid permeation into the stratum corneum. As seen in Fig. 3 the flux from ethanol/cineole approaches that of ethanol alone after about 15 h. Fig. 3b shows an initial burst of high flux in the presence of ethanol/cineole after about 5 h which then decreases with time to that of ethanol alone. The enhancement revert to an effect mainly due to the presence of ethanol. This could be combined with a wash out effect of the terpene by ethanol and/or an lipid degradation of the membrane by ethanol.

The previously reported steady-state penetration of TRH under control conditions was 0.27 ± 0.01 μg/cm^{2}h (Fig. 3b). This corresponds to a permeability coefficient of 5.4×10^{-5} cm/h (Table 1). The presence of 50% ethanol increased the permeation of TRH to 0.83 ± 0.02 μg/cm^{2}h. The addition of 3% cineole in combination with 47% ethanol increased the penetration of TRH to 0.92 ± 0.03 μg/cm^{2}h. Expressed as permeability

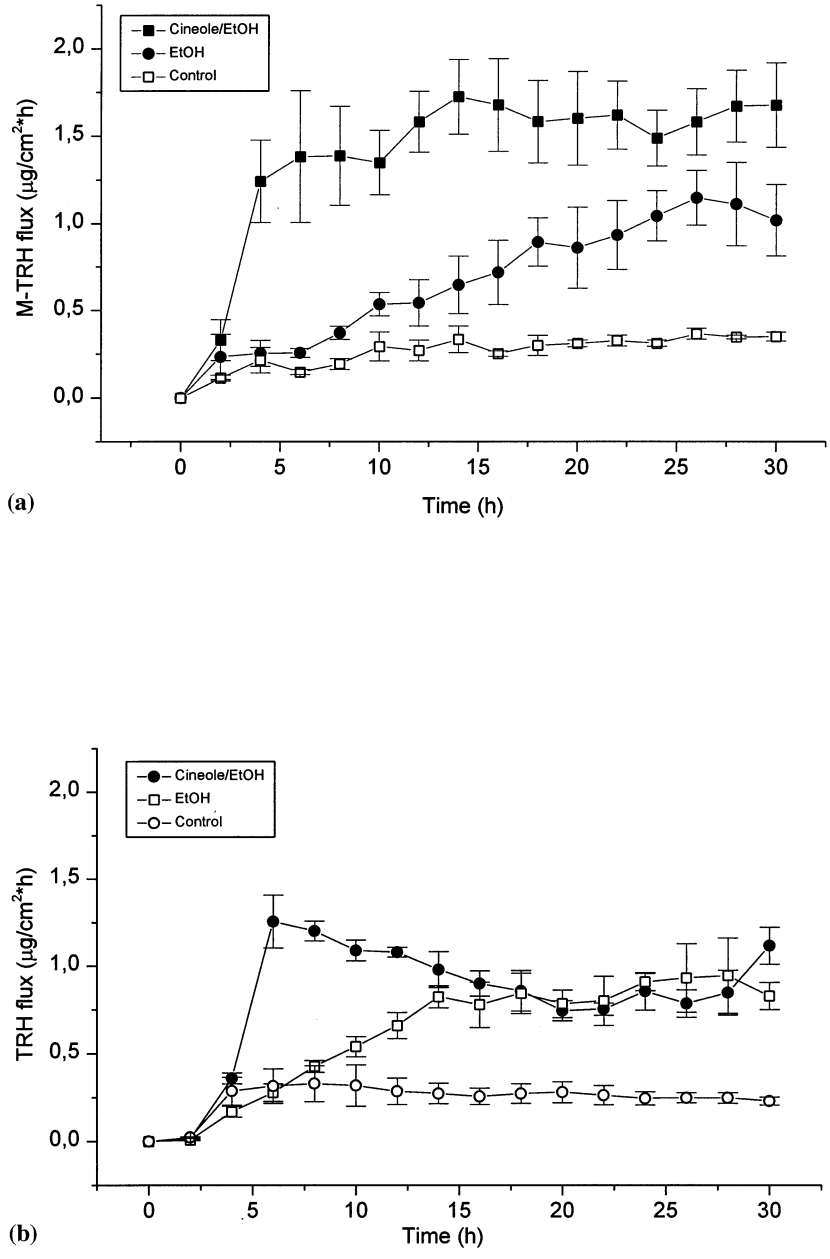

Fig. 3. Penetration of the analogue, M-TRH, and the parental peptide, TRH, through human epidermal membrane. (a) Filled square symbols represent the values for M-TRH flux measured in the presence of 3% cineole in combination with 47% ethanol and 50% PBS ($n = 4$). Filled circles represent the values for the vehicle solution of 50% ethanol/PBS ($n = 3$) and open squares represent the control values for M-TRH ($n = 4$). (b) Filled circle symbols represent the values for TRH flux measured in the presence of 3% cineole in combination with 47% ethanol and 50% PBS ($n = 3$). Open squares represent the values for the vehicle solution of 50% ethanol/PBS ($n = 5$) and open circles represent the control values for TRH ($n = 3$). Values are mean $\pm$ SEM (Data from Magnusson et al., 1997a).

Table 1
The effect of enhancers on the permeability of M-TRH and TRH with different composition of donor solution through hydrated epidermal membranes

Vehicle content	K_p (cm/h $\times 10^5$)[a]	Cumulative amount (μg/cm^2)[b]	EF[c]
M-TRH in PBS	6.8	8.6 ± 1.0	—
TRH in PBS[d]	5.4	7.8 ± 1.7	—
M-TRH with ethanol	20.2	20.4 ± 3.6	3.0
TRH with ethanol[d]	16.6	18.5 ± 2.1	3.1
M-TRH with ethanol/cineole	32.0	41.5 ± 4.9	4.7
TRH with ethanol/cineole[d]	18.4	24.9 ± 1.7	3.4

[a] Permeability coefficient (K_p).
[b] Cumulative amount measured after 30 h, with standard error of mean.
[c] Enhancement factor (EF), the ratio of the drug permeability coefficient in the presence of enhancer to the control permeation of the drug.
[d] Data from Magnusson et al., 1997a.

coefficients, the penetration of TRH with ethanol and TRH in combination with ethanol/cineole, was 16.6 and 18.4×10^{-5} cm/h, respectively (Table 1).

The total amount of penetrated M-TRH as a function of time is illustrated in cumulative plots (Fig. 4). The amount of penetrated M-TRH under control conditions after 30 h was 8.6 ± 1.0 μg/cm^2. The presence of ethanol increased the amount of penetrated M-TRH by approximately three times, to 20.4 ± 3.6 μg/cm^2. In the presence of ethanol and cineole, the amounts of penetrated M-TRH after 30 h was 41.5 ± 4.9 μg/cm^2 (Fig. 4). Statistically significant increase of the total amounts of penetrated M-TRH, were observed in the presence of ethanol/cineole or ethanol alone compared to control conditions ($p \leq 0.05$ Student's t-test). Significantly more M-TRH penetrated in the presence of ethanol/cineole compared to ethanol alone ($p \leq 0.05$ Student's t-test). This difference is, to a large extent, dependent on the reduced lag time observed for the solutions containing cineole. The lag times may be reduced as a consequence of ethanol and cineole increasing diffusivity of the peptide through the membrane. It may also be that there is an effect on the partitioning of the permeant into the membrane from the donor solution. The results for the parental peptide previously reported in part by Magnusson et al. (1997a) are given as a comparison in Table 1. The cumulative amounts for TRH after 30 h under control condition, in the presence of ethanol alone, and in combination with ethanol/cineole were 7.8 ± 1.7, 18.5 ± 2.1, and 24.9 ± 1.7 μg/cm^2, respectively. As seen in Table 1, the enhancement factors for M-TRH and TRH in combination of ethanol and cineole were 4.7 and 3.4, respectively.

4. Discussion

The skin serves as an effective barrier to the external environment. The principal barrier function is contained to the most superficial layer of the epidermis, the stratum corneum. This is clearly demonstrated by the fact that gradual stripping away of the stratum corneum produces a gradual increase in skin permeability (Scheuplein and Blank, 1971). The transdermal route of administration is attractive from various pharmacotherapeutical points of view. The potential advantages of transdermal administration have been well recognized, and several transdermal medications have been developed. For example: scopolamine for motion sickness; nitroglycerin for the treatment of angina pectoris; clonidine for the treatment of hypertension; estradiol for the management of post menopausal symptoms and the treatment of osteoporosis; fentanyl for the treatment of postoperative pain, and nicotine for the reduction of withdrawal symptoms after smoking cessation (Price et al., 1981; Shaw, 1984; Padwick et al., 1985; Rose et al., 1985; Calis et al., 1992).

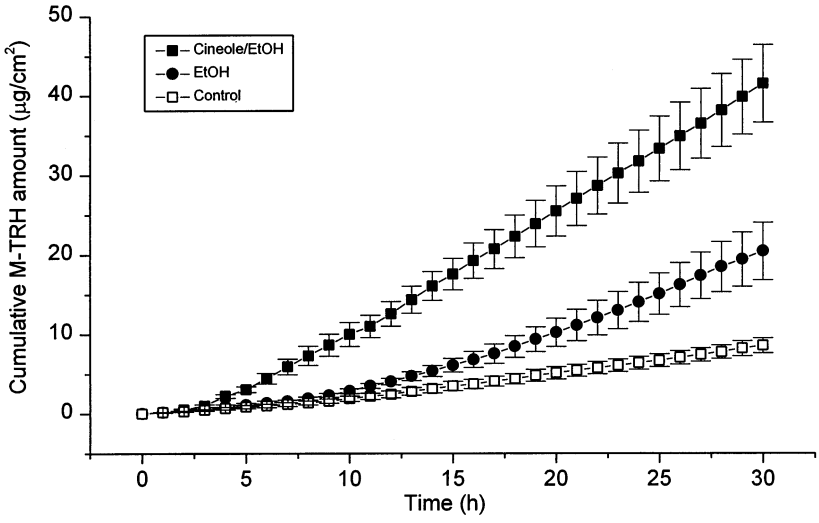

Fig. 4. Cumulative plot of M-TRH penetration as a function of time. Filled square symbols represent the values for flux measured in the presence of 3% cineole in combination with 47% ethanol and 50% PBS. Filled circles represent the values for the vehicle solution of 50% ethanol/PBS and open squares represent the control values for M-TRH. Values are mean ± SEM.

Nonetheless, the characteristics of the stratum corneum barrier have limited the transdermal administration to a relatively small number of drugs having the characteristics that allow for a sufficient rate of penetration through the skin. Attempts to modify the barrier properties of the stratum corneum by the use of enhancers such as terpenes, Azone or the esters of long-chain fatty acids have produced promising new possibilities in transdermal administration (Sugibayashi et al., 1985; Yamane et al., 1995). Penetration enhancers may promote drug permeation across the skin by a variety of mechanisms, and the molecular basis for their actions is becoming clearer (Williams and Barry, 1992). A frequently reported effect is that the enhancers interact reversibly with stratum corneum constituents to disrupt the highly ordered structure and hence facilitate drug diffusion. One effect of the use of the enhancer ethanol, is an increase in the vehicle solubility of the drug.

An increasingly interesting group of substances with potential use as pharmaceuticals are small endogenous peptides (Meyer et al., 1988; Bannerjee and Ritschel, 1989). One of many examples that has already attained clinical use is TRH. Intravenous injection of TRH, the common mode of administration, results in high initial plasma concentrations which is often associated with unpleasant side-effects such as flushing, nausea and cardiovascular reactions (Schurr et al., 1985). By utilizing transdermal delivery such initial peaks in plasma concentrations can be avoided and systemic TRH levels may be maintained within the therapeutic range over a prolonged period of time. To reduce the resistance of the intercellular lipid matrix, we have employed a terpene and ethanol as penetration enhancers. In previously reported studies, the mono-terpene, cineole, has been shown to be an effective penetration enhancer (Williams and Barry, 1991; Magnusson et al., 1997a). In this study 50% ethanol has been used in combination with 3% cineole. This was done to provide a homogenous donor solution where the penetrant could be added concurrently with the terpene. The design allowed the study of the early phase of enhancer-induced changes in the permeability. This is in contrast with many other studies where neat or high concentration of enhancer(s) have been used to pre-treat the skin before the penetrant is applied (Williams and Barry, 1991; Yamane et al., 1995). The comparatively lower enhancement factors obtained in this study may be the result of the low terpene concen-

tration applied and the short duration of the experiment, compared to a long pre-treatment period with neat substance.

The driving force for permeation is the thermodynamic activity of the permeant in the donor solution. The calculation of enhancement factors thus needs to take this into account. The solubility of M-TRH and TRH in the donor solutions was determined in triplicate by liquid scintillation counting. The solutions were saturated with radiolabelled peptide and equilibrated for 24 h in 32.0 ± 0.1°C. The solubility obtained for the tripeptides in the three different compositions was approximately 10 mg/ml. There was no statistically significant difference of solubility between M-TRH and TRH in the different donor solutions. The data from the supplier for the solubility of TRH in water and methanol is 10 mg/ml. According to the solubility data the solution of 5.0 mg M-TRH or TRH per ml can thus be considered as a 50% saturated solution. The maximum increase in flux would for a saturated solution be in the range of two. Since an increase in concentration of the permeants would result in a higher flux, the observed enhancement factors in the range of three to five are interpreted as a true enhancement of flux in the presence of ethanol and ethanol/terpene.

The clinical utility of TRH is hampered by its rapid enzymatic inactivation in the blood as well as by its poor transport over the blood-brain barrier (Metcalf, 1982; Hichens, 1983). The values in this study obtained for penetration of TRH, analyzed by liquid scintillation technique and radio-immunoassay, were very similar. This suggests that in the case of TRH, labelled degradation products do not react in the RIA and therefore do not cause falsely high penetration values. The values of TRH penetration from a PBS donor solution obtained in this study are in agreement with data previously reported by Magnusson et al. (1997a). Many analogues of TRH have been synthesized with the aim of eliciting information on structural requirements of the receptors. The TRH analogue, M-TRH, with a small structural change in the histidine position, pGlu-3-methyl-His^2-Pro amide, have a high potency and stimulates the release of TSH seven to eight times from the pituitary compared to the parental tripeptide. The difference in chemical structure of the peptides are illustrated in Fig. 1. Our results show that the transdermal penetration of the analogue in the presence of enhancers is in the same order of magnitude or greater than that of the parental peptide. As the two studies are performed on epidermal membranes from different individuals, the possible inter-individual variations may partly cause the differences in penetration rate. However, the measurements of water penetration and TRH from a PBS donor solution indicates that the membranes from the two sources have similar properties. If the true value of M-TRH is higher compared to TRH, it is probably a reflection of the differences in chemical properties. A simple measurement of octanol/PBS partition coefficient was performed using shake-flask procedure (Hansch and Leo, 1995). The partition coefficients obtained for M-TRH and TRH were 7.7 and 6.0×10^{-3} (pH 7.4), respectively. This indicates that M-TRH is slightly more lipophilic than TRH and may therefore have a greater penetration potential. One of many examples of methylation that leads to an increase in lipophilicity is morphine, codeine and heroin. Each addition of a methyl group increases the transport over the blood-brain barrier (Kosterlitz and Waterfield, 1975).

In vitro penetration studies of TRH have previously been investigated by using excised mouse or human skin (Burnette and Marrero, 1986; Moss and Bundgaard, 1990). These investigators could not detect any measurable amount of penetrated TRH. The limit of detection used in those studies was about 0.5 μg/ml (HPLC analysis). This could account for their inability to detect penetrated TRH. We have successfully achieved detectable levels for transdermal penetration of both TRH and its analogue, through human skin by using liquid scintillation technique and/or RIA. We have also demonstrated the feasibility of achieving transdermal delivery of the two peptides at therapeutically relevant amounts by using penetration enhancers such as ethanol and cineole. In our study, 5.0 mg/ml of M-TRH in combination with ethanol and cineole results in a flux of 1.60 ± 0.02 μg/cm^{2}h. If a patch with an active area of 20 cm^2

was used, this could allow for the delivery of approximately 630 μg of M-TRH over 24 h. For comparison, the corresponding amount for TRH in the presence of ethanol/cineole was 400 μg over 24 h. This is in the same order of magnitude as the TRH dosage used clinically (500 μg) and is given to humans by infusion or injection during 24 h (Mitsuma and Nogimori, 1984; Lampe et al., 1989). The delivered amounts of the two peptides do not represent the maximum obtainable values since the composition of the vehicle has not been optimized. However, the results from this study clearly demonstrate the ability of enhancers to effectively modify stratum corneum permeability and to achieve successful transdermal transport of thyrotropin-releasing hormone and its analogue through human epidermal skin.

In conclusion, this study provides evidence that, even in the absence of enhancers other than water, a peptide can cross the human stratum corneum in vitro at a measurable rate. These investigations have also determined the passive permeation of the tripeptide M-TRH across human skin and found the result to be in agreement with those earlier reported for the parental peptide. The values obtained for TRH-penetration, analyzed by liquid scintillation technique and RIA, were very similar. This suggests that in the case of TRH, no degradation of the peptide occurs. The results furthermore suggest that in the presence of the enhancers, the peptide flux significantly increases to a level where, at least theoretically, the delivery of a therapeutically relevant dose is possible. Our results show that the transdermal penetration of the analogue in the presence of enhancers is in the same order of magnitude or greater than that of the parental peptide. Further studies should be undertaken to investigate this aspect and also investigate whether biological response can be elicited by transdermally-delivered peptide.

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References

Bannerjee, P.S., Ritschel, W.A., 1989. Transdermal permeation of vasopressin. II. Influence of Azone on in vitro and in vivo permeation. Int. J. Pharm. 49, 199–204.

Burnette, R.R., Marrero, D., 1986. Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin. J. Pharm. Sci. 75, 738–743.

Calis, K.A., Kohler, D.R., Corso, D.M., 1992. Transdermally administered fentanyl for pain management. Clin. Pharm. 11, 22–36.

Hansch, C., Leo, A., 1995. The hydrophobic parameter: Measurement and calculation. In: Heller, S.R. (ed.), Exploring QSAR: Fundamentals and Applications in Chemistry and Biology. American Chemical Society, Washington, pp. 97–121.

Hichens, M., 1983. A comparison of thyrotropin-releasing hormone with analogs: Influence of disposition upon pharmacology. Drug Metab. Rev. 14, 77–98.

Horita, A., Carino, M.A., Lai, H., 1986. Pharmacology of thyrotropin-releasing hormone. Annu. Rev. Pharmacol. Toxicol. 26, 311–332.

Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human stratum corneum. Arch. Dermatol. 88, 702–705.

Kosterlitz, H.W., Waterfield, A.A., 1975. In vitro models in the study of structure-activity relationships of narcotic analgesics. Annu. Rev. Pharmacol. Toxicol. 15, 29–47.

Lampe, T.H., Veith, R.C., Plymate, S.R., Risse, S.C., Kopeikin, H., Cubberley, L., Raskind, M.A., 1989. Pressor, norephinephrine, and pituitary responses to two TRH doses in Alzheimer's disease and normal older men. Psychoneuroendocrinology 14, 311–320.

Magnusson, B.M., Runn, P., Karlsson, K., Koskinen, L.-O.D., 1997a. Terpenes and ethanol enhance the transdermal permeation of the tripeptide thyrotropin releasing hormone in human epidermis. Int. J. Pharm. 157, 113–121.

Magnusson, B.M., Runn, P., Koskinen, L.-O.D., 1997b. Terpene-enhanced transdermal permeation of water and ethanol in human epidermis. Acta Derm. Venereol. 77, 264–267.

Metcalf, G., 1982. Regulatory peptides as a source of new drugs-the clinical prospects for analogues of TRH which are resistant to metabolic degradation. Brain Res. Rev. 4, 389–408.

Meyer, R.B., Kreis, W., Eschbach, J., O'Mara, V., Rosen, S., Sibalis, D., 1988. Successful transdermal administration of therapeutic doses of a polypeptide to normal human volunteers. Clin. Pharmacol. Ther. 44, 607–612.

Mitsuma, T., Nogimori, T., 1984. Changes in plasma thyrotropin-releasing hormone, thyrotropin, prolactin and thyroid hormone levels after intravenous, intranasal or rectal administration of synthetic thyrotropin-releasing hormone in man. Acta Endocrinol. 107, 207–212.

Moss, J., Bundgaard, H., 1990. Prodrugs of peptides. 7. Transdermal delivery of thyrotropin releasing hormone (TRH) via prodrugs. Int. J. Pharm. 66, 39–45.

Padwick, M.L., Endacott, L., Whitehead, M.I., 1985. Efficacy, acceptability, and metabolic effects of transdermal estradiol in the management of postmenopausal women. Am. J. Obstet. Gyneol. 152, 1085.

Price, N.M., Schmitt, L.G., McGuire, L.G., Shaw, J., Trobough, G., 1981. Transdermal scopolamine in prevention of motion sickness at sea. Clin. Pharmacol. Ther. 29, 414.

Rose, J.E., Herskovic, J.E., Trilling, Y., Yarvik, M.E., 1985. Transdermal nicotine reduces cigarette craving and nicotine preferences. Clin. Pharmacol. Ther. 38, 450.

Scheuplein, R.J., Blank, J.H., 1971. Permeability of the skin. Physiol. Rev. 51, 702–747.

Schurr, W., Knoll, B., Ziegler, R., Anders, R., Merkle, H.P., 1985. Comparative study of intravenous, nasal, oral and buccal TRH administration among healthy subjects. J. Endocrinol. Invest. 8, 41–44.

Shaw, J.E., 1984. Pharmacokinetics of nitroglycerin and clonidine delivered by the transdermal route. Am. J. Heart J. 108, 217.

Sowers, J.R., Hershman, J.M., Pekary, A.E., Nair, M.G., Baugh, C.M., 1976. Effect of *N*-methyl-thyrotropin releasing hormone on the human pituitary thyroid axis. J. Clin. Endocrinol. Metab. 43, 471–748.

Sugibayashi, K., Hosoya, K., Marimoto, Y., Higuchi, W.I., 1985. Effect of the absorption enhancer, Azone, on the transport of 5-FU across the hairless rat skin. J. Pharm. Pharmacol. 37, 578–580.

Ward, D.J., Finn, P.W., Griffiths, E.C., Robson, B., 1987. Comparative conformation-activity relationship for hormonally- and centrally-acting TRH analogues. Int. J. Peptide Protein Res. 30, 263–274.

Williams, A.C., Barry, B.W., 1992. Skin absorption enhancers. Crit. Rev. Ther. Drug Carrier Syst. 9, 305–353.

Williams, A.C., Barry, B.W., 1991. The enhancement index concept applied to terpene penetration enhancers for human skin and model lipophilic (oestradiol) and hydrophilic (5-fluorouracil) drugs. Int. J. Pharm. 74, 157–168.

Yamane, M.A., Williams, A.C., Barry, B.W., 1995. Effects of terpenes and oleic acid as skin penetration enhancers towards 5-fluorouracil as assessed with time; permeation, partitioning and differential scanning calorimetry. Int. J. Pharm. 116, 237–251.