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Prodrugs of peptides. 7. Transdermal delivery of thyrotropin-releasing hormone (TRH) via prodrugs

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Summary

The feasibility of providing transdermal delivery of the tripeptide TRH (pGlu-His-Pro-NH_2) was examined using the prodrug approach. The prodrugs studied were the *N*-isobutyloxycarbonyl and *N*-octyloxycarbonyl derivatives formed by reacting TRH with the appropriate chloroformates at its imidazole moiety. These derivatives are quantitatively converted to TRH by spontaneous or non-specific esterase-catalyzed hydrolysis, but are resistant towards degradation by the TRH-specific pyroglutamyl aminopeptidase serum enzyme. Diffusion experiments in vitro using human skin samples showed that, whereas TRH and *N*-isobutyloxycarbonyl-TRH did not penetrate the skin to any measurable extent, the *N*-octyloxycarbonyl derivative showed a high penetrating capacity. By applying this prodrug derivative in an aqueous solution (pH 6.0) at a concentration of 5%, a steady-state flux of 16 μg TRH/h per cm^2 was observed while a 5% solution in propylene glycol gave a flux of 3.6 μg TRH/h per cm^2. Essentially all of the prodrug penetrated was present in the receptor phase as TRH. The study demonstrates the feasibility of achieving transdermal delivery of TRH based on the favourable skin penetration properties of the *N*-octyloxycarbonyl-TRH prodrug which in turn are attributed to its combination of high water solubility and lipophilicity.

Introduction

Thyrotropin-releasing hormone (TRH, pGlu-L-His-L-Pro-NH_2) is the hypothalamic peptide that regulates the synthesis and secretion of thyrotropin from the anterior pituitary gland. Since its discovery in 1969, TRH has been shown to have not only a variety of endocrine and central nervous system-related biological activity, but also potential as a drug in the management of various neurologic and neuropsychiatric disorders including depression, brain injury, acute spinal trauma and schizophrenia (for reviews, see Jackson, 1982; Metcalf, 1982; Griffiths, 1985, 1986, 1987; Horita et al., 1986; Loosen, 1988; Metcalf and Jackson, 1989).

The clinical utilization of the neuropharmacological properties of TRH is, however, greatly hampered by its rapid metabolism and clearance as well as by its poor access to the CNS (Metcalf, 1982; Hichens, 1983; Griffiths, 1987; Loosen, 1988). Following parenteral administration in man TRH shows a plasma half-life of only 6–8 min (Bassiri and Utiger, 1973; Morley et al., 1979; Duntas et al., 1988; Iversen, 1988) which mainly is due to rapid enzymatic degradation of the peptide

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in the blood, in particular by the so-called TRH-specific pyroglutamyl aminopeptidase serum enzyme (PAPase II) (Bauer, 1988; Emerson, 1989; Møss and Bundgaard, 1990). This enzyme catalyzes the hydrolysis of TRH at the pGlu-His bond yielding pyroglutamic acid and His-Pro-NH_2 (Fig. 1). The same degradation can also be effected by virtue of the less specific pyroglutamyl aminopeptidase PAPase I which occurs in many different tissues such as liver, kidney and brain but not in the blood (Abraham and Podell, 1981; Bauer, 1988; Wilk, 1989). The lipophilicity of TRH is very low (Bundgaard and Møss, 1990) and this may be a primary reason for the limited ability of the peptide to penetrate the blood-brain barrier (Nagai et al., 1980; Banks and Kastin, 1985).

We have recently reported that these problems of rapid enzymatic inactivation and poor lipophilicity of TRH may be overcome by bioreversible derivatization of the peptide (Bundgaard and Møss, 1989, 1990). The derivatives developed are *N*-alkoxycarbonyl derivatives of TRH formed by *N*-acylating the imidazole group of the histidine residue with various chloroformates. These derivatives are totally resistant to cleavage by the TRH-inactivating serum enzyme, but are readily bioreversible as the parent TRH is formed quantitatively from the derivatives by spontaneous hydrolysis or by plasma esterase-catalyzed hydrolysis (Scheme 1). Besides protecting the parent TRH against inactivation in plasma and hence being potentially useful to prolong the duration of action of TRH in vivo, the *N*-alkoxycarbonyl prodrug derivatives possess greatly increased lipophilicity relative to TRH as assessed by octanol-buffer partition experiments (Bundgaard and Møss, 1990). This property may render the prodrug forms more capable of penetrating the blood-brain barrier or various other biomembranes than the parent peptide.

The objective of the present study was to determine whether these prodrugs could be utilized to develop a transdermal delivery system of TRH. This route of administration would seem attractive from various pharmacotherapeutical points of view. Thus, intravenous injection of TRH which is the commonly used mode of administration is often associated with unpleasant side-effects such as flushing, nausea and cardiovascular reactions due to the initial high plasma concentrations (Anders and Merkle, 1983; Schäfgen et al., 1983; Schurr et al., 1985). By transdermal delivery such plasma peaks may be avoided and systemic TRH levels be maintained within the therapeutic range over a prolonged period of time. A prerequisite for the development of a transdermal delivery system of TRH is, however, that the peptide is capable of penetrating the skin at a sufficiently high rate and is not degraded during the percutaneous absorption. Burnette and Marrero (1986) have previously shown that TRH does not penetrate excised mouse skin to any measurable extent, but that the transdermal transport can be enhanced by iontophoresis. The poor skin permeability of TRH can most likely be ascribed to its very low lipophilicity. TRH is weakly basic with a pK_a value of 6.25 (Grant et al., 1972) but although it is almost fully

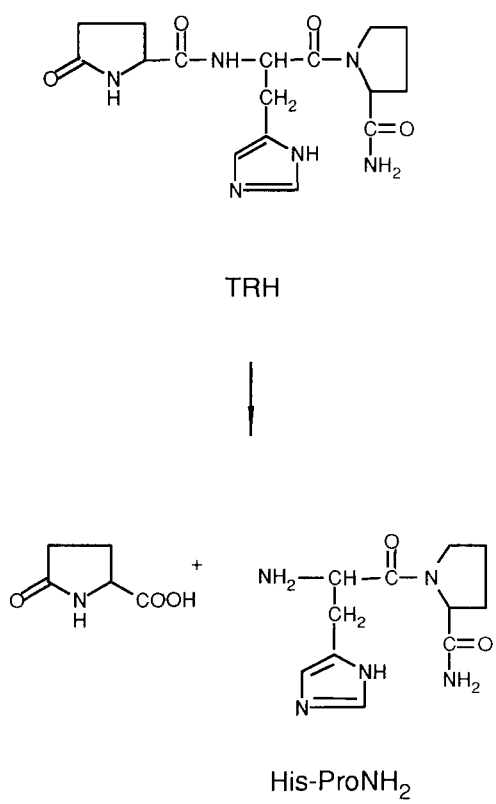
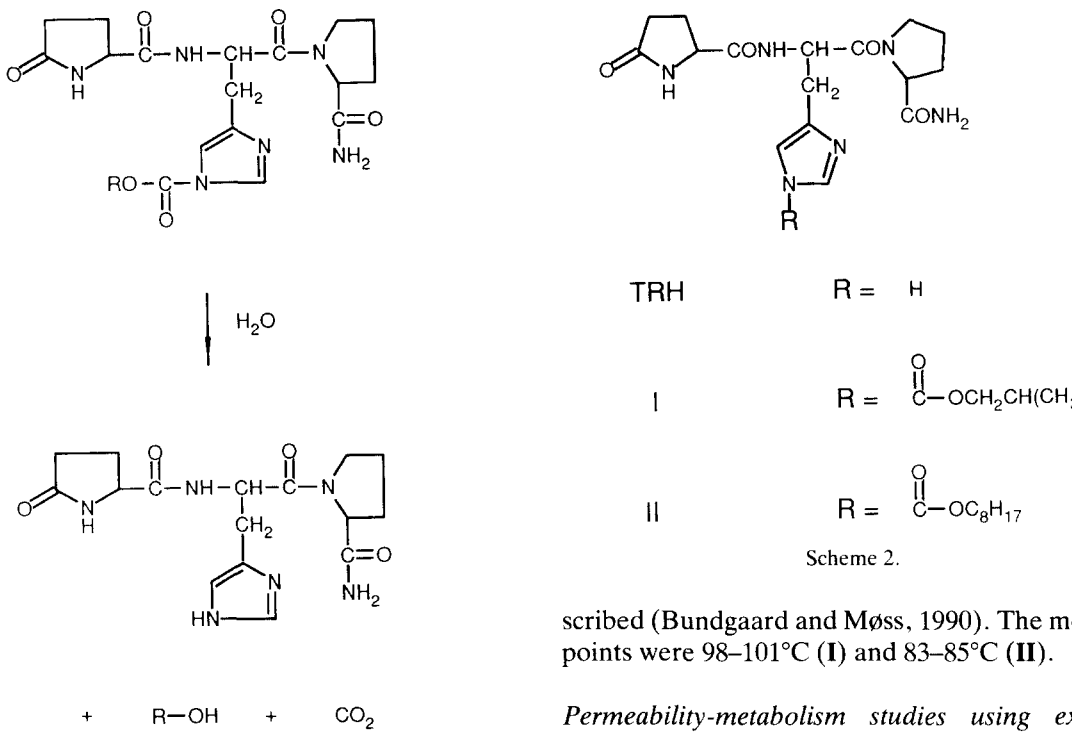


Fig. 1. Hydrolysis of TRH by virtue of pyroglutamyl aminopeptidases to pyroglutamic acid and L-histidyl-L-prolineamide.



Scheme 1.

unionized at pH 7.4, its log P value is only −2.46 (P represents the partition coefficient between octanol and aqueous buffer of pH 7.4) (Bundgaard and Møss, 1990).

In the present study, the feasibility of achieving transdermal delivery of TRH via the prodrug approach was evaluated by diffusion experiments in vitro using human skin samples. The prodrugs studied were the *N*-isobutyloxycarbonyl (**I**) and *N*-octyloxycarbonyl (**II**) derivatives of TRH. These derivatives show greatly increased lipophilicities relative to TRH, their log P values being −0.44 (**I**) and 1.88 (**II**), respectively (Bundgaard and Møss, 1990).

Materials and Methods

Chemicals

TRH was obtained from Carlbiotech A/S, Copenhagen. The *N*-alkoxycarbonyl-TRH derivatives **I** and **II** were prepared as previously described (Bundgaard and Møss, 1990). The melting points were 98–101°C (**I**) and 83–85°C (**II**).

Scheme 2.

Permeability-metabolism studies using excised human skin

Whole abdominal human skin obtained under autopsy was used. The skin was stored at −18°C and was allowed to thaw gradually at room temperature before use. All subcutaneous fat was removed and the skin cut into pieces. The excised skin was mounted in open diffusion cells of the same type as those used by Franz (1975); they have an available diffusion area of 0.70 cm^2. The dermal side of the skin was exposed to the receptor medium (5 ml of 0.05 M isotonic phosphate buffer solution of pH 7.2) which was stirred magnetically and kept at a constant temperature of 37°C with a circulating water bath.

The compounds were applied as 5% w/v solutions in 0.05 M phosphate buffer (final pH being 6.0) or propylene glycol (100 or 200 μl). At appropriate intervals samples of 1 ml were removed from the receptor phase and replaced with fresh buffer. The samples were immediately analyzed for TRH and prodrug content by HPLC as described below. The permeation studies of each compound in each vehicle were done in triplicate.

Stability studies

The stability of TRH and the *N*-octyloxycarbonyl derivative (**II**) was examined in the presence of whole human skin. Pieces of the skin were slightly macerated in 0.05 M phosphate buffer of pH 7.40, the 'homogenate' concentration being 20%. The compounds were incubated at 37°C in this mixture at an initial concentration of 2×10^{-4} M. At various intervals samples of 500 μl were withdrawn and added to 500 μl of a 2% solution of zinc sulphate in 50% v/v methanol in order to deproteinize the samples. After immediate mixing and centrifugation at 13000 rpm for 3 min, 20 μl of the clear supernatant was analyzed by HPLC as described below.

HPLC analysis of TRH and its prodrugs

TRH and its prodrugs **I** and **II** were determined by HPLC using a system consisting of a Kontron 420 HPLC pump, a Kontron 432 LC detector operated at 215 nm and a Rheodyne 7125 injection valve with a 20-μl loop. A reversed-phase column (33 × 4.6 mm) packed with Supelcosil LC-8-DB (3-μm particles) and protected with a Supelguard LC-8-DB column (from Supelco Inc., U.S.A.) was used for the determination of the prodrugs **I** and **II**. The solvent systems used were acetonitrile-0.1% v/v phosphoric acid (20:80 v/v) (compound **I**) or (40:60 v/v) (compound **II**), the flow rate being 1 ml min^{-1}. For the analysis of TRH a ChromSep column (100 × 4.6 mm) packed with Microspher C-18 (3-μm particles) and supplied with a Chrompack guard column was eluted at ambient temperature with a mobile phase consisting of methanol-0.1% v/v phosphoric acid (2:98 v/v), the flow rate being 1 ml min^{-1}. It was ensured that the various degradation products of TRH (Møss and Bundgaard, 1990) did not interfere with the determination of TRH using this HPLC procedure. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

Results and Discussion

Excised human skin was used to examine the percutaneous absorption of TRH and the two *N*-alkoxycarbonyl-TRH prodrug derivatives **I** and **II**. The compounds were applied in the form of 5% w/v aqueous solutions (pH 6.0) or propylene glycol solutions. The value of pH 6 was chosen since the *N*-alkoxycarbonyl derivatives show maximal stability at this pH (Bundgaard and Møss, 1990). The pK_a value for the derivatives is 2.7 (Bundgaard and Møss, 1990) and therefore, the compounds are totally unionized at pH 6. The solubility of the compounds in both the pH 6 buffer and propylene glycol was so high (> 20% w/v) that it was not practically possible (due to the limited supply of compounds) to use suspensions which generally is to be preferred in order to keep a constant driving force for diffusion and to provide the maximum flux attainable.

In the case of both TRH and the *N*-isobutyloxycarbonyl derivative (**I**) no measurable amounts of TRH or intact prodrug could be detected in the receptor phase during diffusion experiments lasting up to 200 h. The limit of detection of TRH and the prodrug was about 0.5 μg ml^{-1}. The failure of TRH to penetrate human skin is in accordance with the results obtained by Burnette and Marrero (1986) using excised nude mouse skin.

In contrast, the more lipophilic *N*-octyloxycarbonyl derivative (**II**) readily penetrated human skin. The results obtained with this prodrug are shown in Fig. 2 in which the cumulative amounts (in μmol) of TRH measured in the receptor phase divided by the surface area of the diffusion cell are plotted against the time of sampling. Essentially all of the prodrug penetrated through the skin was present as TRH in the receptor phase. Thus, at sampling times of 50–70 h, 5–8% was present in the form of intact prodrug whereas this figure dropped to 2–3% at sampling times of 100–140 h. The half-life of hydrolysis of compound **II** to TRH in the phosphate buffer used as the receptor phase was determined to be 20 h at 37°C. Therefore, it can be calculated that part of the prodrug conversion might have taken place after penetration by spontaneous hydrolysis.

The steady-state fluxes obtained from the slopes of the linear portions of the plots in Fig. 2 were 0.045 ± 0.006 μmol TRH/h per cm^2 for the 5% aqueous buffer solution and 0.010 ± 0.002 μmol

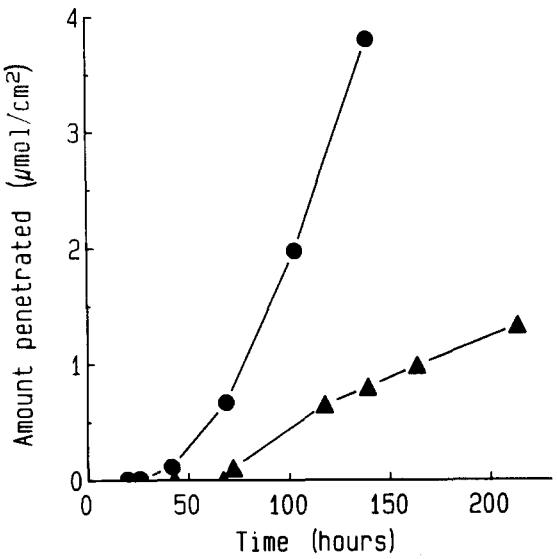


Fig. 2. Permeability of the *N*-octyloxycarbonyl derivative of TRH (**II**) through human skin as amount of TRH appearing in the receptor phase as a function of time from 5% solutions of **II** in 0.05 M phosphate buffer of pH 6.0 (●) and propylene glycol (▲). The data represent the mean values obtained from 3 diffusion cell studies.

TRH/h per cm^2 for the 5% solution in propylene glycol. These figures correspond to 16 μg TRH/h per cm^2 and 3.6 μg TRH/h per cm^2, respectively.

The good skin penetration behaviour observed for the *N*-octyloxycarbonyl prodrug derivative can most likely be attributed to its combination of high water solubility and lipophilicity which combination is an important determinant of flux across skin (Roy and Flynn, 1989; Sloan, 1989). Both TRH and the *N*-isobutyloxycarbonyl derivative are also highly water soluble, but their lipophilicity in terms of octanol-buffer partition coefficients is much lower than that of compound **II** (Bundgaard and Møss, 1990).

Stability studies with TRH and the prodrug **II** in a 20% human skin homogenate revealed a fairly high rate of degradation. Under the experimental conditions used both compounds degraded according to first-order kinetics. The *N*-octyloxycarbonyl derivative was found to be degraded with half-lives of 4–6 h at 37°C depending on the skin samples used. The reaction taking place was entirely hydrolysis at the *N*-alkoxycarbonyl moiety to yield TRH. With various skin samples half-lives of 7–15 h were observed for the degradation of TRH. Although being appreciable, these rates of degradation are much lower than those in human plasma solutions. Thus, the half-life for hydrolysis of compound **II** to TRH is 24 min in human plasma at 37°C whereas TRH is degraded with a half-life of 9.4 min (Bundgaard and Møss, 1990). The significance of the breakdown of TRH observed under these incubation conditions in relation to the penetration studies is difficult to access. In the iontophoretic transport studies of TRH across excised nude mouse skin no degradation of TRH during the diffusion was observed (Burnette and Marrero, 1986).

The feasibility of obtaining transdermal delivery of TRH via the prodrug approach can be assessed by comparing the daily doses of TRH usually used via parenteral administration with those obtainable by transdermal delivery or by taking the pharmacokinetic approach based on the clearance rate of the drug and its therapeutic plasma concentration (Knepp et al., 1987). If the area of the patch for transdermal delivery is 20 cm^2 and if a flux of 16 μg/h per cm^2 is used, it would be possible to deliver 320 μg TRH/h or 7.6 mg over 24 h. This amount is in the same range (0.5–10 mg) as that often given by infusion or injection of TRH during 24 h (see, e.g., Sawin et al., 1977; Ogashiwa and Takeuchi, 1979; Mitsuma and Nogimori, 1984; Lampe et al., 1989). It should be recognized that this flux does not represent the maximally obtainable value since the prodrug was not applied in the form of a saturated solution, and no optimization studies concerning the type of vehicle, use of penetration enhancers etc. were done.

In conclusion, this study demonstrates, apparently for the first time, that the prodrug approach may be useful for enhancing the delivery of a peptide across human skin. As discussed elsewhere (Barry, 1986; Meyer et al., 1988; Bodde et al., 1989) transdermal administration of highly potent peptides offers a number of potential advantages. The prodrug approach used in the present study and based on transforming a hydrophilic peptide to a bioreversible derivative with a higher biphasic solubility may probably be applicable to various other small peptides besides TRH.

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