# ON THE CONFORMATION OF THYROTROPIN RELEASING HORMONE

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## 1. Introduction

The thyrotropin releasing hormone (TRH) of the hypothalamus which is the tripeptide pyroglutamyl-histidylprolinamide (pGlu–His–Pro–$NH_2$) was the first hypothalamic neurohormone to be structurally elucidated and synthesized [1,2]. Since TRH is a small molecule with great biological importance a large number of analogs of the hormone have been synthesized and studied in various assay systems [3–5]. These studies have been very important to elucidate the structure–function relationship and the biochemical mechanism of action of TRH. Another approach to the structure–function problem of TRH is to define the shape of the molecule and the relationship between conformation and biological activity. Recently several papers have appeared which are concerned with conformations of TRH [6–10].

This report describes the biological activities of four tri-peptides, pGlu–Leu–Pro–$NH_2$ (*1*), pGlu–Leu–Pro–$OCH_3$ (*2*), pGlu–Thi–Pro–$NH_2$ (*3*) and pGlu–Thi–Pro–$OCH_3$ (*4*) (Thi = β-(2-thienyl)-L-alanine). The chemical properties of these peptides appear to add information about the conformation of TRH when their biological potencies are evaluated together with the recent findings of Burgess et al. [11] on the low energy conformations of TRH.

## 2. Materials and methods

Peptides *1–4* have been synthesized by classical methods in solution as previously described by Sievertsson et al. [12]. The assays for TSH release in vivo in mice have been performed using the $T_3$-TRH method of Bowers et al. [13,14].

To measure the in vitro effect of TRH and its analogs, 2 pituitaries obtained from 20-day-old female Sprague–Dawley rats (Charles River Laboratory) were incubated at 37°C in 1 ml of lactated Ringer's glucose (1 mg/ml) medium (Travenol Laboratories) in 10 ml Teflon beakers in a Dubnoff shaker. Pituitaries were incubated for a total of 6 hr. Medium was removed each hour for RIA of TSH and fresh medium was added. After two pre-incubation periods (1 hr each), the peptides were added each hour. Each analog was measured in duplicate by the NIAMDD method of Parlow (personal communication). TSH is reported in equivalents of NIAMDD rat TSH–RP–1 which has a biological potency of 0.22 USP (Bovine) TSH Units/mg by the McKenzie Assay. (The nomenclature of the amino acids and peptides are following the IUPAC-IUB recommendations 1972).



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## 3. Results and discussion

The data on the thyrotropin (TSH) induced release in vivo in mice are presented in table 1. Leu$^2$–TRH (*1*) has about 2% of the activity of TRH while Thi$^2$–TRH (*3*) show up to 30% hormonal activity. However, none of the peptides have a dose–response curve parallel to that of TRH. The Me-esters, □–Glu–Leu–Pro–$OCH_3$ (*2*) and Glu Thi–Pro–$OCH_3$ (*4*) were previously found inactive, when tested in vivo for release of TSH and prolactin together with analogs *1,3* and additional six new tripeptides [12]. The results of the TSH induced release by the analogs when assayed in vitro are summarized in table 2. In the in vitro system Thi$^2$–TRH (*3*) has as much as 77% of the activity of TRH, while Leu$^2$–TRH (*2*) has similar potency as in the in vivo study or about 3%. The Me-esters, *2* and *4* are essentially inactive (table 2).

After comparisons between the p*K*a values and biological potencies of TRH and analogs of TRH Grant et al. [6] suggested a preferred conformation of TRH having a hydrogen bond between the imidazol $\pi$N and the histidyl $\alpha$N–H. From results of NMR studies Fermandjian et al. [7] proposed a similar conformation for the His moiety. Burgess et al. [11] more recently suggested a somewhat different conformation of TRH from results obtained on conformational energy calculations of TRH and several of its analogs. Their data imply that in the low energy forms the central His moiety is in an extended conformation, which means that the imidazole ring is directed away from the other atoms. They also suggest that a seven membered hydrogen bonded ring

Table 1
Assay of 2 tripeptides by the $T_3$-method in vivo in mice

| Tripeptide | Dose in ng | $I^{125}$ Δ cpm[a] | Rel. activity % |
|---|---|---|---|
| TRH | 1 | 2.306 | |
| | 3 | 5.332 | 100 |
| | 9 | 11.178 | |
| □Glu–Leu–Pro $NH_2$ (*1*) | 10 | 240 | |
| | 30 | 606 | 2 |
| | 100 | 4.110 | |
| □Glu–Thi–Pro–$NH_2$ (*3*) | 10 | 5.110 | 30 |
| | 100 | 10.007 | |
| Saline | – | 470 | – |

[a] Each result is the mean value of 5 mice.

Table 2
In vitro TSH releasing activity of tripeptides[a]

| Peptides | Dose ng/ml medium | Activity ng TSH/ml medium/hr | Activity/ Dose |
|---|---|---|---|
| TRH | 0.3 | 30.000 | $1.0 \times 10^5$ |
| □Glu–Leu–Pro–$NH_2$ (*1*) | 10.0 | 33.250 | $3.3 \times 10^3$ |
| □Glu–Leu Pro–$OCH_3$ (*2*) | $5.0 \times 10^4$ | 7.500 | $1.5 \times 10^{-1}$ |
| □Glu–Thi–Pro–$NH_2$ (*3*) | 0.3 | 23.000 | $7.7 \times 10^4$ |
| □Glu–Thi–Pro–$OCH_3$ (*4*) | $5.0 \times 10^4$ | 23.000 | $4.6 \times 10^{-1}$ |

[a] Two hourly pre-incubation and 4 hourly incubation periods during peptide stimulation. Activity = mean results of 2 consecutive stimulations periods. Mean TSH release during pre-incubation periods ranged 3000–7000 ng TSH/ml medium/hr.

is formed between the C-terminal N–H and the oxygen in the peptide bond between His and Pro [11]. The latter also has been proposed by Fermandjian et al. [7].

Replacement of the imidazole ring of the His residue with a lipophilic aliphatic carbon side chain as in peptide *1* not only changes the over all charge of the molecule but also excludes any possibility of forming an internal hydrogen bond with the side chain. Also $Thi^2$–TRH (*3*) having the essentially neutral aromatic thienyl side chain in the second position probably is uncharged at a physiological pH. Nevertheless *1* and *3* are remarkably potent analogs of TRH suggesting that for hormonal activity the side chain in position two is not confined into a single conformation due to a side chain backbone interaction. The necessity of a side chain has been evidenced by the inactivity of $Gly^2$–TRH [12].

The importance of aromaticity and special sterical requirements of the second amino acid of TRH might explain the differences in potencies between *1* and *3* (tables 1 and 2). The lack of basicity in $Thi^2$–TRH (*3*) is probably the reason why this analog is less potent than TRH. We have previously proposed the same interpretation to explain the decreased activity of $Phe^2$–TRH, an analog having up to 10% of the activity of TRH [15].

If the inactivity of the Me-esters, *2* and *4*, reflect the inability of these analogs to form the seven membered ring between the C-terminal N–H and the previous peptide bond [7,11] then this requirement is extremely important for activity. The modification of TRH to its corresponding Me-ester will greatly diminishes its hormonal activity [3–5]. However, it cannot be excluded, as pointed out by Burgess et al. [11], that such a modification might cause a sterically unfavourable interaction at the receptor site, 'since no large changes in the conformation of the rest of the structure would be expected'. To be considered is that the tetrapeptide pGlu–His–Pro–Gly–$NH_2$ has 30% of the activity of TRH when tested in vivo in mice [16].

Just like TRH peptides *1* and *3* increased the serum levels of prolactin when tested in monkeys [12].

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