(terminal nerve) which mediates responses to sexual pheromones[9]. The innervation of the retina by fibers of the terminal nerve suggests that the GnRH acts as a transmitter in this retinopetal system[10]. All these data confirm the important role of this system in sexual behaviour as has been described in mammals[11]. The connection of the cell bodies described in the preoptic region with the pituitary gland suggests that this center acts more directly on the gonadotrophic function. Further studies are necessary to determine the relationship between the two centers and the multifunctional actions of GnRH.

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TRH analogue with C-terminal thioamide group. Synthesis, receptor binding, TSH-releasing activity and α-MSH-releasing activity[1]

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Summary. A new TRH analogue containing a C-terminal thioamide group was synthesized. This peptide was shown to have receptor-binding affinity, and TSH- as well as α-MSH-releasing activities very similar to native TRH.
Key words. [Prot^3]TRH; receptor-binding affinity; TSH-releasing activity; α-MSH-releasing activity; thiopeptide.

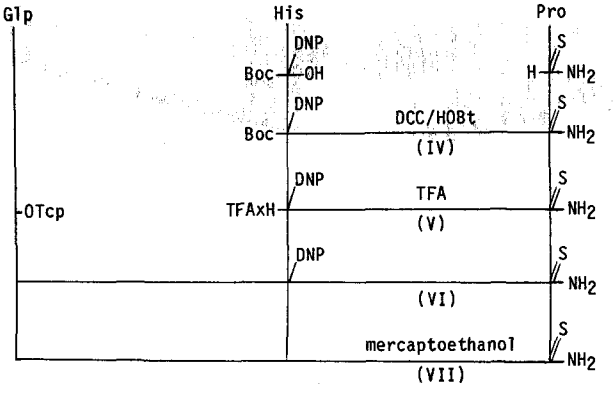
Thyrotropin-Releasing Hormone (TRH), identified as L-pyroglutamyl-L-histidyl-L-proline amide[2,3], stimulates in mammals the release of both thyrotropin[4,5] and prolactin[5] whereas, in amphibians, TRH was found to be a potent melanotropin-releasing factor[6]. In this communication we wish to report the synthesis of L-pyroglutamyl-L-histidyl-L-proline thioamide ([Prot^3]TRH) (**VII**) from proline thioamide (**III**) applying Lawesson's Reagent (LR) as thionation agent[7]. The biological part of our report describes the evaluation of the receptor-binding affinity as well as the α-MSH- and TSH-releasing activities of this TRH thioanalogue.

Chemistry. L-Proline thioamide (**III**) was synthesized by the route shown in scheme 1.

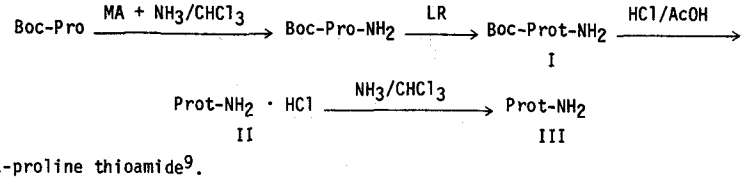
The structural proofs of compounds **I**–**III** were based on elemental analysis, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

The [Prot^3]TRH (**VII**) was synthesized as shown in scheme 2.

Compound **III** was coupled with Boc-His(DNP) by means of the DCC/HOBt method[10] to give **IV** in 75% yield. Acidolytic removal, using TFA, of the Boc-protecting group from **IV** involved some difficulties, apparently due to the formation of the thioanalogue of the corresponding diketopiperazine (L-thioprolyl-L-histidyl). Diketopiperazine formation from carboxy-terminal proline dipeptide esters is well documented[11], but such a rapid cyclization of a dipeptide amide is less common[12]. The extent of this side reaction could be minimized, however, by



Scheme 2. Preparation of L-pyroglutamyl-L-histidyl-L-proline thioamide[9].



Scheme 1. Preparation of L-proline thioamide[9].

The biological activities of [Prot^3]TRH[9]

Compound	Receptor-binding affinity	Releasing activity	
	IC_{50} (nM) (n)	TSH (%) (n)	α-MSH (%)
TRH	32 ± 2.8 (8)	100 ± 9.8[a] (6)	100 ± 5.6
[Prot^3]TRH	49 ± 5.0 (4)	98.7 ± 12.9[a] (6)	104 + 13.6

[a]TSH releasing activity in vivo 20 min after i.p. injection of 5 µg/kg b.wt in female rats. Mean ± SE of n experiments (receptor-binding) or animals (TSH-release).

lowering the TFA temperature to 0 °C, reducing the time of exposure to TFA and immediately coupling with Glp-OTcp. The crude **VI** was chromatographed on silica (70% yield, calculated on the amount of **IV** used) to give a product containing only a trace of a highly polar impurity. The removal of the DNP group from **VI** was accomplished with mercaptoethanol to give the final product **VII**, which could be purified by chromatography on silica and Sephadex G-10 (80% yield). The correct structure of **VII** was proved by ^{1}H-NMR and ^{13}C-NMR spectroscopy, mass spectrometry, amino acid and elemental analyses. The homogeneity of **VII** was demonstrated by TLC and HPLC. HPLC showed it to be completely free from the corresponding D-His diastereoisomer. On the other hand, another synthesis of **VII** from the N-terminal dipeptide[13] and **III**, (2+1 coupling) gave a product containing 21% of the LDL form as estimated with this method.

Stability of [Prot^3]TRH. The possible degradation of **VII** at different pH values was examined by HPLC. The peptide is fully stable in 5% acetic acid. At pH 7.4 about 10% decomposition products were formed in 4 days (1–2% of TRH). In water, the half-life of **VII** was estimated to 8–9 days. All stability tests were performed at room temperature.

Biology. The binding affinity of **VII** for TRH receptors in the membrane fraction of the rat anterior pituitary was determined[14] and the result is presented in the table. The α-MSH-releasing activity was studied in perfused frog neuromediate lobes[15]. The TSH-releasing activity of the analogue in the rat pituitary is also presented.

As seen from these results, there is a good correlation between the three biological tests. [Prot^3]TRH appears to be approximately equipotent with synthetic TRH in vitro and in vivo.

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9 Abbreviations: The symbols for the amino acids and peptides are in accordance with the Recommendations 1983 of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (Eur. J. Biochem. *138* (1984) 9). DCC – dicyclohexylcarbodiimide, DNP – 2,4-dinitrophenyl, MA – mixed anhydride with isobutyl chloroformate, OTcp – 2,4,5-trichlorophenyl, TFA – trifluoroacetic acid, HOBt – N-hydroxybenzotriazole, LR – 2,4-bis-(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide (Lawesson's Reagent), IC_{50} – the concentration of drug inhibiting specific binding by 50%.
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Babesia bovis: the effect of acute inflammation and isoantibody production in the detection of babesial antigens

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Summary. Immunoblots of *Babesia bovis* antigen contain dominant antigens which react not only with antisera to *B.bovis* but with sera from naive calves recovering from an acute inflammatory reaction. It seems likely these antigens are from the host rather than the parasite.

Key words. Babesia; antigen; inflammation; immuno blotting.

The intra-erythrocytic protozoan parasite *Babesia bovis* causes a severe and often fatal infection in susceptible cattle. However, cattle surviving initial infection are immune from reinfection for at least four years and probably for the remainder of their lifespans[1]. A proven and probably the main mediator of protection is humoral antibody as serum and IgG from immune cattle can protect susceptible cattle from an otherwise fatal challenge[2]. Moreover, protection can be induced by vaccinating susceptible cattle with fractions from disintegrated infected erythrocytes[3]. Studies at this laboratory have centred round a systematic fractionation of infected erythrocytes and subsequent vaccination trials with selected fractions. The final product of such a procedure is likely to be a cocktail vaccine of a few protective antigens. Immunoblotting[4] has been used to target for the latter but results described in this manuscript question the usefulness of this technique and suggest that the major antigen(s) detected by blotting are of host rather than parasite origin.

Methods. Calves, 3–6 months of age, were obtained from areas known to be free of the tick vector, *Boophilus microplus*. On arrival at the laboratory they were tested for *B.bovis* or its