Frontiers of Hormone Research

Thyrotrophin Releasing Hormone

1

Volume Editors:
R. Hall, Newcastle upon Tyne
I. Werner, Basel
H. Holgate, London
Series Editor:
M. Marois, Paris

S. Karger
Basel · München · Paris · London · New York · Sydney

Workshop Conference on Thyrotrophin Releasing Hormone, Basel, April 2–3, 1971

Thyrotrophin Releasing Hormone

Sponsored by F. Hoffmann-La Roche & Co. Ltd., Basel

Edited by
Reginald Hall
University of Newcastle, Department of Medicine,
Royal Victoria Infirmary, Newcastle upon Tyne

Inna Werner
Department of Clinical Research,
F. Hoffmann-La Roche & Co. Ltd., Basel

Harold Holgate
Department of Clinical Research,
Roche Products Ltd., London

134 figures, 28 tables

S. Karger · Basel · München · Paris · London · New York · Sydney · 1972

S. Karger · Basel · München · Paris · London · New York · Sydney
Arnold-Böcklin-Strasse 25, CH–4000 Basel 11 (Switzerland)

Printed in Switzerland by Buchdruckerei Tanner & Bosshardt AG, Basel

List of Participants

Chairman

Prof. R. Hall, University of Newcastle, Department of Medicine, Royal Victoria Infirmary, Newcastle upon Tyne (England)

Vice-Chairman

Prof. H. Studer, Medizinische Klinik der Universität, Inselspital, Bern (Switzerland)

Participants

Doz. Dr. S. Almqvist, Endokrinolog. Klin., Karolinska Sjukhuset, Stockholm (Sweden)
Dr. L. Baschieri, Istituto di Medicina del Lavoro della Università di Pisa, Pisa (Italy)
Prof. C. Beckers, Université de Louvain, Centre de Médecine Nucléaire, Cliniques Universitaires St. Pierre, Louvain (Belgium)
Dr. G.M. Besser, St. Bartholomew's Hospital, London (England)
Dr. J.-L. Codaccioni, Hôpital de la Conception, Marseille (France)
Prof. P. Franchimont, Université de Liège, Hôpital de Bavière, Liège (Belgium)
Prof. T.R. Fraser, Royal Postgraduate Medical School, Department of Medicine, London (England)
Dr. L. Frische, Diakonissehuset Skt. Lukas Stiftelsen, Hellerup (Denmark)
Dr. J. Herrmann, Medizinische Hochschule Hannover, Dept. Innere Medizin, Abteilung für Klinische Endokrinologie, Hannover (Germany)
Doz. Dr. R. Höfer, Allg. Krankenhaus der Stadt Wien, 2. Medizinische Universitäts klinik, Wien (Austria)
Dr. Ph. Jaquet, Faculté de Médecine de Marseille, Hôpital de la Conception, Marseille (France)
Dr. J. Ketelslegers, Université de Liège, Hôpital de Bavière, Liège (Belgium)
Prof. A. Labhart, Medizinische Universitätsklinik, Kantonsspital Zürich, Zürich (Switzerland)

Dr. N.F. Lawton, Institute of Nuclear Medicine and Institute of Clinical Research, The Middlesex Hospital, London (England)
Doz. Dr. P.O. Lundberg, Academic Hospital, Uppsala (Sweden)
Prof. R. Mornex, Hôpital de l'Antiquaille, Service d'Endocrinologie, Lyon (France)
Dr. A. von zur Mühlen, Medizinische Klinik und Poliklinik der Universität Göttingen, Göttingen (Germany)
Dr. B.J. Ormston, University of Newcastle, Department of Medicine, Royal Victoria Infirmary, Newcastle upon Tyne (England)
Dr. F. Piva, Università degli Studi, Istituto di Farmacologia e di Terapia, Milano (Italy)
Prof. A. Prader, Kinderspital, Zürich (Switzerland)
Dr. G. Rothenbuchner, Universität Ulm, Medizinisch-Naturwissenschaftliche Hochschule, Departement für Innere Medizin, Ulm (Germany)
Dr. K. Scholz, Chirurgische Universitätsklinik, Innsbruck (Austria)
Dr. H. Steiner, Department of Experimental Medicine, F. Hoffmann-La Roche & Co., Ltd., Basel (Switzerland)
Dr. R.O. Studer, Department of Chemical Research, F. Hoffmann-La Roche & Co., Ltd., Basel (Switzerland)
Prof. A. Vanotti, Clinique médicale universitaire, Hôpital Cantonal, Lausanne (Switzerland)
Dr. W. Waldhäusl, I. Medizinische Klinik der Universität, Wien (Austria)
Prof. A. Walser, Medizinische Universitäts-Poliklinik, Basel (Switzerland)
Dr. J. Weeke, Kommunehospital, Århus (Denmark)
Dr. Inna Werner, Department of Clinical Research, F. Hoffmann-La Roche & Co., Ltd., Basel (Switzerland)
Dr. Jane Wood, Department of Medicine, Gardiner Institute, Western Infirmary, Glasgow (Scotland)

Welcoming Address

On behalf of the Research Department of F. Hoffmann-La Roche & Co., Ltd., I welcome you most sincerely to this Workshop Conference. The aim of this meeting is to form an opinion on TRH, the first synthetic releasing hormone to be investigated clinically. Clearly the synthesis of releasing hormones offers the research scientist and the clinician new and valuable possibilities, first in diagnosis and perhaps eventually in therapy. With your help we will try to discuss the results obtained to date and speculate on the possibilities of a clinical application. Allow me to thank you for participating in this conference which I now have pleasure in asking Professor HALL to open.

Dr. Inna Werner
Department of Clinical Research
F. Hoffmann-La Roche & Co., Ltd., Basel

Contents

List of Participants VII
Welcoming Address IX

Introduction

Hall, R.: Opening Remarks 2
Studer, R.O.: Chemistry of TRH 4
Piva, F. and Steiner, H.: Bioassay and Toxicology of TRH. Animal Studies 11
Franchimont, P.: TSH Immunoassay. Assay Factors Affecting TSH Levels 22

Clinical Effects of TRH

Almqvist, S.: Clinical Side Effects of TRH 38
Ormston, B.J.: Clinical Effects of TRH on TSH Release after i.v. and Oral Administration in Normal Volunteers and Patients with Thyroid Disease 45
Fraser, T.R.: Effects of TRH on TSH Release in Patients with Pituitary-Hypothalamic Disease 76
Lawton, N.F.: Effects of TRH on Thyroid Hormone Release 91
Studer, H.: Effects of TRH on Thyroidal Radioiodine Uptake 114

Effects of TRH on and Interaction with Other Pituitary Hormones

Besser, G.M.: Effects of TRH on and Interaction with Other Pituitary Hormones 128

Effects of TRH on Other Metabolic Parameters

Mühlen, A. von zur: Effects of TRH on Other Metabolic Parameters 150

Future Implications of TRH

Beckers, C.: Immunoassay of TRH and its Problems 170
Diagnostic Applications of TRH. General Discussion 176
Therapeutic Applications of TRH. General Discussion 187

Conclusions

Hall, R.: Concluding Remarks 192

Introduction

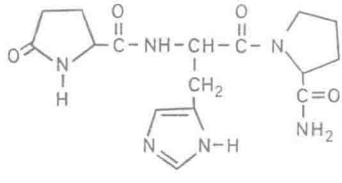
Front. Hormone Res., vol. 1, pp. 2–3 (Karger, Basel 1972)

Opening Remarks

R. HALL

University of Newcastle, Department of Medicine, Royal Victoria Infirmary, Newcastle upon Tyne

We shall start the programme with the chemistry of thyrotrophin-releasing hormone, to be followed by the bioassay and toxicology of TRH. Following this Prof. FRANCHIMONT will talk about TSH immunoassay. The particular type of assay used will greatly influence the results obtained with TRH. Dr. ALMQVIST will talk on the clinical side-effects of TRH and we hope to have some information on the mechanism of these side-effects from Dr. ORMSTON. Dr. ORMSTON will talk on the effect of TRH on TSH release in control subjects and in patients with thyroid disease. Prof. FRASER will describe the use of TRH in patients with disease of the pituitary and hypothalamus. Dr. LAWTON will discuss the effects of TRH on various parameters of thyroid hormone release, in particular thyroxine and triiodothyronine levels in the serum. Prof. H. STUDER will talk on the effects of TRH on thyroidal-radioiodine uptake using TRH as a stimulant for endogenous TSH. Dr. BESSER will discuss the interaction of TRH with stimuli causing ACTH and growth hormone release. Dr. VON ZUR MÜHLEN will talk on the effects of TRH on



Structure of TRH.

other metabolic parameters apart from the pituitary hormones. Prof. BECKERS will discuss some of the problems of TRH immunoassay. Finally there will be a session on the diagnostic and therapeutic applications of TRH.

Thyrotrophin-releasing hormone is a simple tripeptide, pyroglutamyl-histidyl-proline-amide blocked at both ends of the molecule. In this Conference we should keep to the nomenclature suggested by SCHALLY, referring to this synthetic tripeptide as thyrotrophin-releasing hormone. Dr. R.O. STUDER from Roche, Basel, will now talk on the chemistry of this particular releasing hormone.

Front. Hormone Res., vol. 1, pp. 4–10 (Karger, Basel 1972)

Chemistry of TRH

R.O. STUDER

Department of Chemical Research
F. Hoffmann-La Roche & Co., Ltd., Basel

It is a special pleasure for me to talk about the chemistry of TRH to this audience, which has given so fruitful a continuation of our chemical work. But I do this only with some hesitation, because one and a half years after the elucidation of the structure and the total synthesis of TRH the excitement and the accent have definitely shifted away from the chemistry to the clinical aspects and the chemist should from now on stand behind the scene of such a Workshop Conference.

You will be aware that the early work on the releasing factors goes back as far as the early 1930's. By 1955 enough convincing evidence had accumulated for it to be concluded that the hypothalamus dominates the functions of the anterior pituitary gland and that this control must be exerted by way of some hypothetical substances released into the portal blood. The purification of these substances, however, has met unusual difficulties, and this for two main reasons: (1) the development of a sufficiently simple and sensitive bioassay; (2) the extremely low concentration of the releasing factors in the hypothalamus.

Despite these difficulties enough evidence has now accumulated to indicate that each anterior pituitary hormone is controlled by a factor in the hypothalamus (table I). Here we have a summary of the releasing factors and you see that the term releasing factors originally proposed by SAFFRAN has been substituted by SCHALLY and his group for hormone, and at the present time you have 2 nomenclatures 'releasing factors' e.g., thyrotrophin releasing factor, luteinizing hormone releasing factor and on the other hand you have thyrotrophin releasing hormone and luteinizing hormone releasing hormone. A clear nomenclature will have to await its time. As Prof. HALL said before, he prefers the term 'releasing hormone' and this coincides with our

Table I. Nomenclature of some hypothalamic hormones

Present name		Proposed name	
Hypothalamic factor	Abbreviation	Hypothalamic hormone	Abbreviation
Corticotrophin-releasing factor	CRF	Corticotrophin-releasing hormone	CRH
Luteinizing hormone-releasing factor	LRF or LH-RF	Luteinizing hormone-releasing hormone	LH-RH or LRH
Follicle-stimulating hormone-releasing factor	FSH-RF	Follicle-stimulating hormone-releasing hormone	FSH-RH or FRH
Thyrotrophin-releasing factor	TRF	Thyrotrophin-releasing hormone	TRH
Growth hormone-releasing factor or somatotrophin-releasing factor	GRF or SRF	Growth hormone-releasing hormone or somatotrophin-releasing hormone	GRH or SRH
Prolactin-inhibiting factor (mammals)	PIF	Prolactin release-inhibiting hormone	PRIH
Melanocyte-stimulating hormone (MSH) release inhibiting factor	MIF	MSH-release-inhibiting hormone	MRIH

[After SCHALLY, A.V. *et al:* Recent Progr. Hormones. Res. *24:* 497, 1968].

opinion. As you can see our programme goes under the name 'Thyrotrophin Releasing Hormone' or 'TRH' and all communications to be presented will use the name TRH.

That the hypothalamus is responsible for regulating the release of thyrotrophin (TSH) from the anterior pituitary by means of a neurohormonal agent was postulated on the basis of physiological experiments by HARRIS, GREER, D'ANGELO and REICHLIN in the early 1960's. HARRIS, 1963, GUILLEMIN and JUTISZ, in 1963 and REICHLIN, in 1964, observed that extracts from the median eminence increase thyroid function in mice and rabbits. There were mainly 2 groups deeply involved in working up hundreds of thousands of hypothalami for TRH, namely the group of GUILLEMIN in Houston working with ovine glands and the group of SCHALLY in New Orleans working with glands of porcine origin.

In January 1969, Dr. GUILLEMIN's group had isolated 1 mg of highly-purified TRH from 270,000 sheep glands. The total recovery in terms of activity was about 20%. From this one can estimate that each hypothalamus

contains about 20 ng of TRH. By assuming that the human hypothalamus contains about the same amount one can calculate that the total amount of human TRH existing at this very moment in the whole world adds up to about 100 g, and this is just the amount you have all been using for your clinical studies. Happily enough this material was of synthetic origin. GUILLEMIN's material behaved like a polypeptide and yielded after total hydrolysis 3 amino acids, namely histidine, proline and glutamic acid, the amino acids accounting for 81% of the total weight. It was at this moment, in January 1969, when the existing loose contact between Roche and Dr. GUILLEMIN was, of course, immediately tightened and the 2 groups acted as a single team.

It was decided to attack the elucidation of the structure from 2 sides.

On the one side to obtain from the precious and scarce natural TRH as much information as possible without using up the whole amount. On the other hand to synthesize all possible tripeptide combinations with the 3 amino acids and use them as model compounds (table II).

With the 3 amino acids, glutamic acid, histidine and proline you can synthesize 6 possible tripeptides if you assume that all peptide bonds are α-connected. We have first the 2 histidines at the N-terminal-side of the tripeptide and then you can vary the proline and the glutamic acid. You can put the proline at the amino end then vary the histidine and the glutamic acid or you can begin with the glutamic acid and vary the histidine and the proline. All 6 tripeptides were synthesized but none had any biological activity whatsoever. However, in the meantime Dr. GUILLEMIN confirmed earlier work that in TRH there is no free N-terminal amino group and, therefore, this group had to be protected in some way. Nature uses for the purpose of protecting amino groups of amino acids usually acetic acid residues like, in α-MSH, where you have at the N-terminus acetyl-serine. Therefore, all 6 tripeptides were submitted to acetylation conditions. After this treatment, to

Table II. The 6 possible tripeptides built from glutamic acid, histidine and proline, having all α-connected peptide bonds

H–His–Pro–Glu–OH
H–His–Glu–Pro–OH
H–Pro–His–Glu–OH
H–Pro–Glu–His–OH
H–Glu–His–Pro–OH
H–Glu–Pro–His–OH

our surprise, one, and only one, of the 6 model compounds showed a slight biological activity qualitatively indistinguishable from the TRH, namely the chain which had the sequence glu-his-pro. All the others were inactive.

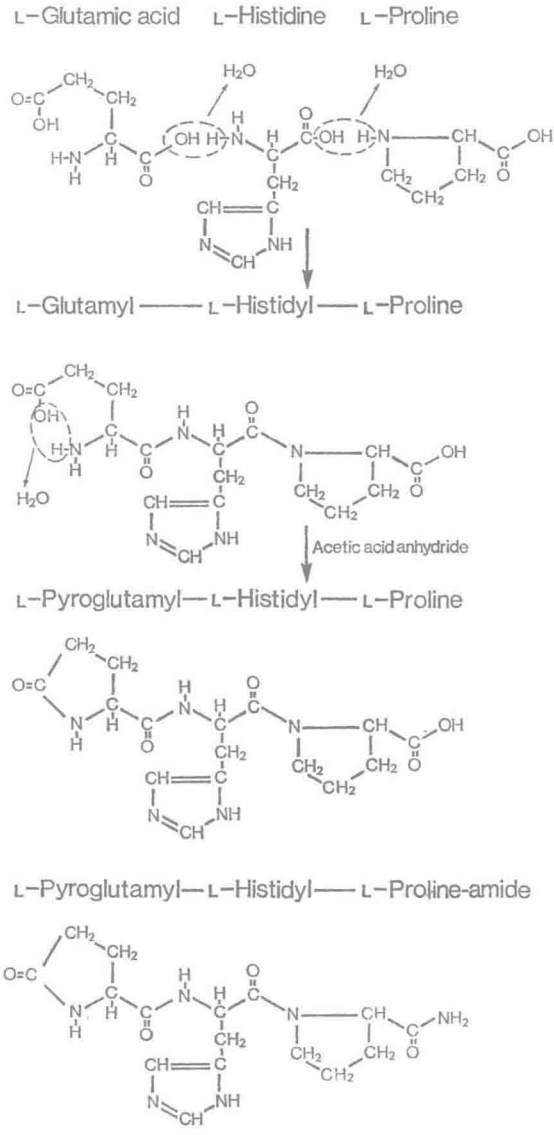


Fig. 1. Structure elucidation and synthesis of ovine TRH.

Therefore, a large amount of this material was prepared by the same methods.

The acetylation mixture contained about 6 different substances, they were all separated by physicochemical methods and tested for biological activity. From the 6 compounds in the acetylation mixture one, and only one was active. The structure of this active substance was deduced to be *pyro-glutamyl-histidyl-proline* (fig. 1).

We have here in the beginning 3 amino acids, glutamic acid, histidine and proline which are condensed to the tripeptide glutamyl-histidyl-proline. This then is submitted to acetylation conditions and we expected that it will be acetylated at the free amino group. But this was not the case, the α-amino group had condensed with the γ-carboxyl of the glutamic acid to give the pyroglutamic acid or the pyrolidone carboxylic acid.

From the differences in specific biological activities and electrophoretic and chromatographic behaviour between pyroglu-his-pro and the natural ovine TRH it was evident that the 2 were not identical but very closely related. The physicochemical methods indicated that natural TRH was more basic than our tripeptide. A more basic, that is less acidic compound, can be obtained by protecting the free carboxyl group of proline. Nature usually does this by forming an amide. Therefore, the next move was to transform the pyroglu-his-pro via its methylester into the amide. This compound was found to be fully identical with the natural ovine TRH. At about the same time SCHALLY's group found an identical structure for porcine TRH, and very recently they found good evidence that also human TRH also has the same structure. What now are the features of this molecule? (fig. 2).

First of all, it is an unbelievably small molecule which has a surprisingly high and specific biological activity, but I would remind you this is not so unusual when you compare it for instance with gastrin, where the C-terminal tetrapeptide of gastrin has the full biological activity of the whole molecule. The shortness of this peptide, however, will greatly reduce the possibilities for species differences, which you always have in very large peptides.

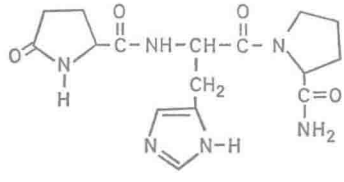


Fig. 2. Structure of TRH.

The most striking feature in this molecule is the accumulation of ring structures. We have a pyroglutamic acid, we have the imidazole ring of the histidine and we have the proline ring. Of these rings the pyroglutamic acid seems somewhat unusual, but it has been found before in nature, for instance in gastrin we have a pyroglutamic acid at the N-terminus, and also in certain peptides isolated from amphibian skin. It seems that pyroglutamic acid is also at the end of the luteinizing hormone releasing hormone. These ring structures greatly reduce access to the amide-bonds and, therefore, they reduce greatly the danger of enzymatic attack. This is one of the reasons why TRH is so stable and why it is possible to use it orally which is a very unusual feature for polypeptides.

Unfortunately we do not yet have much information about the conformation, mainly because TRH is not yet in crystalline form. Therefore, this is about all I have to tell you about the chemistry and I think it is time now to shift to the more interesting clinical aspects of TRH.

Author's address: Dr. R.O. STUDER, Chemische Forschungsabteilung, F. Hoffmann-La Roche & Co., AG, Grenzacherstrasse 124, *CH–4000 Basel* (Switzerland)

Discussions

HALL: Thank you for your contribution Dr. STUDER. Are there any points for discussion?

FRASER: Could I ask one question merely? You say that it is very resistant to acid, does this apply to acid-alkaline changes and to heat too?

R.O. STUDER: Under normal hydrolyzing conditions (6N HCl, 24 h at 110°C) you can hydrolyze the compound into its amino acids. But under normally-encountered conditions it is a very stable compound.

WALDHÄUSL: Can this compound be easily attacked by some naturally-occurring enzymes? Let us say if you incubate this material human plasma, how fast does it deteriorate, what is the half-life time under *in vitro* conditions?

R.O. STUDER: There is a paper by SCHALLY dealing with the inactivation of TRH in plasma and it seems that this inactivation is not only due to enzymes, but it is also due to adsorption. He made some studies of the adsorption of TRH on human albumin and here he observed quite rapid inactivation. It also seems to be at the present time also an adsorption effect, but I remind you that there exists an enzyme which splits off pyroglutamic acid. However we don't know if this enzyme is present in plasma.

WALDHÄUSL: This adsorption to plasma is it irreversible or can it be reversed?