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(Citation)
The Kobe journal of the medical sciences,18(1):15-19

(Issue Date)
1972-03

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100488990

Kobe J. Med. Sci. 18, 15-19, March 1972

NEUROENDOCRINE CONTROL OF THE PITUITARY TSH SECRETION

VI Effect of Long Term Administration of Synthetic Thyrotropin-Releasing Hormone (TRH) on Pituitary-Thyroid Function in Rats

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Indexing Words
TRH;
hypothalamus;
TSH pituitary;
thyroid function

Masahiro SAKODA, Hidetaro MORI, Makoto ŌTSUKI, Tsuneo FUKUDA and Shigeaki BABA. *Neuroendocrine Control of the Pituitary TSH Secretion. VI. Effect of Long Term Administration of Synthetic Thyrotropin-Releasing Hormone (TRH) on Pituitary-Thyroid Function in Rats.* Kobe J. Med. Sci. 18, 15-19, March 1972—— Introductory observations were performed on the effect of long term administration of synthetic thyrotropin-releasing hormone (TRH), pyroglutamil-histidyl-proline amid, upon pituitary-thyroid function in rats. High serum PBI levels as well as no increment of resting plasma TSH levels were observed in rats treated with synthetic TRH of 32 days long administration. High PBI levels continued for additional 38 days after final injection of TRH. Metabolic variations of protein and fat in these rats were similar to those of the patient with hyperthyroidism.

INTRODUCTION

Thyrotropin-releasing hormone has synthesized by Folkers,[10] and its biological action in animals[3,5] as well as clinical use[4,9,12,13] has been studied. These studies have revealed that synthetic TRH evokes rapid plasma TSH rise in animals or men accompanying serum PBI increase, and that the cases with pituitary failure or thyroid disorder do not show any TSH response to TRH. However, those studies dealt with transient response, then question arises concerning the effect of long term administration of synthetic TRH. This paper dealt with introductory observations on pituitary thyroid function and metabolic variations in the rats treated with synthetic TRH for a long term.

MATERIALS AND METHODS

Male rats of S-D, JCL strain in 5 weeks age (weighing 110±14g) were used for recipient of synthetic TRH for a long term. Using oriental chow for diet, rats were kept 10 hrs. for lighting and 14 hrs. for dark at the room temperature of 23°C

Received for publication February 12, 1972
Authors' names in Japanese: 佐古田雅弘, 森顕太郎, 大槻真, 福田恒夫, 馬場茂明

±2. Female mice of D-D strain (weighing 15±1 g) were adopted for TSH-bioassay, feeding with low iodine diet and distilled water. The TRH used in this study was synthesized by the joint work of Dr. Sakoda and Daigo Nutritive Chemicals Ltd. following the method of Gillessen[11] with purification using gel filtration, and presented as pyroglutamyl-histidyl-proline-amide acetate. TRH activity of this preparation was determined with *in vivo* and *in vitro* methods. Plasma TSH was assayed after McKenzie[16] with purification using gel filtration. Serum PBI was measured by autoanalyzer technique. Blood chemical analysis was performed on the following indices: Serum total cholesterol,[20] phospholipid,[14] triglyceride,[6] NEFA,[8] urea nitrogen,[17] nonprotein nitrogen, total protein, protein fraction, blood grucose.[18] Synthetic TRH was administered i. p. once a day (at 10:00 A.M.) for 32 days. Biological and chemical analyses were performed 24 hrs. after final TRH injection with 4 hrs. previous fasting. Rats were divided into 3 groups receiving 100, 1,000 μg/kg body weight of TRH and saline respectively. A half of each group lasted in non-treatment for additional 38 days. The statistical significances of the effect of TRH and saline were assessed by performing Student "t" Test on the difference of values in individual experiments.

RESULTS

1. *Effect of a long term administration of TRH on pituitary-thyroid function.*

A significant increase of serum PBI was observed in the rats treated with 100 μg/kg of TRH for 32 days. The PBI increase was also observed in the groups receiving 1,000 μg/kg of TRH, but there was relative variance of PBI value in this group. The rats lasted in non-treatment for 38 days after TRH injection of 32 days duration showed a significant PBI increase in the group receiving 1,000 μg/kg of TRH, while some rats treated with 100 μg/kg of TRH also showed PBI increment. There was no difference of resting plasma TSH levels between the rats treated with TRH for 32 days (24 hrs. after final injection) and control rats (Table 1).

Table 1 Changes in serum PBI and plasma TSH of rats treated with synthetic TRH for a long term.

Treatment	No. of rat	Serum PBI (μg/dl)	P	TSH ^{131}I release from thyroid gland of mice % of initial	P
32 days administration of TRH					
100 μg/kg	5	4.0±0.06*	<0.025	102.1±15	N. S.
1000 μg/kg	6	4.0±0.20	<0.25	96.8± 8.2	N. S.
Saline	7	3.6±0.12	—	89.6±11.2	—
38 days after treatment with 32 days administration of TRH					
100 μg/kg	6	4.3±0.2	<0.25	92.5± 8.4	N. S.
1000 μg/kg	7	5.1±0.5	<0.025	90.9±10.5	N. S.
Saline		3.8±0.2	—	88.4± 7.9	—

*Mean±S.E.

2. *Metabolic changes in the rats treated with synthetic TRH for a long term*

There could not be observed any difference of fasting blood sugar between the groups receiving TRH for a long term and control group. Significant low levels of serum total cholesterol were observed in the groups treated with 100 or 1,000 μg/kg of TRH for 32 days. Serum phospholipids were also lower in the group receiving TRH than in the control group. Mean values of serum triglyceride were relatively high in the TRH treated rats though there was some individual difference. The rats receiving TRH for a long term showed significant low levels of serum, urea-N, non-protein-N and α_1-globulin. There could not be observed any difference of serum NEFA, total protein, A/G ratio and the other protein fraction between the TRH treated rats and the control rats.

Table 2 Blood chemical analysis of rats treated with synthetic TRH for 32 days.

	32 days administration of TRH				Saline
	100 μg/kg	P*	1000 μg/kg	P*	
Blood glucose (mg/dl)	139±6**	—	125±5	—	127±3
Total cholesteral (mg/dl)	74±3	—	70±2	<0.005	78±4
Phospholipid (mg/dl)	109±4	—	103±5	<0.025	126±9
Triglyceride (mg/dl)	64±13	—	67±8	—	61±14
NEFA (meq/l)	0.81±0.11	—	0.89±0.06	—	1.04±0.16
Urea N (mg/dl)	18.2±0.5	<0.005	16.3±0.6	<0.001	22.4±0.5
Non-protein N (mg/dl)	26.8±0.8	—	23.0±0.7	<0.001	30.4±2.3
Total protein (g/dl)	5.70±0.08	—	5.80±0.15	—	5.52±0.22
α_1-globulin (%)	18.7±0.2	<0.005	18.2±0.5	<0.005	21.0±0.4
A/G ratio	0.85±0.05	—	0.83±0.04	—	0.82±0.06

* Versus saline treatment

— represents "not significant"

** Mean±S. E. of 7 rats

DISCUSSION

Influence of a long term administration of synthetic TRH upon pituitary-thyroid function is remained obscure. The authors have revealed that continuous TRH administration evoked hyperthyroid state in rats at the end of 32 days long injection. It is of interest that hyperthyroid state continued for additional 36 days without TRH administration. Although an attempt to develop the experimental hyperthyroidism in animals has been made using TSH preparation, only a transient hyperthyroid state was observed in rats at 8-10 days after continuous TSH injection and then metabolic rate (0_2 consumption) gradually declined over next 3-4 weeks.[2] It is speculated that some inhibiting factors, such as anti-TSH antibody, may be in the serum of these TSH treated rats. Therefore, it is thought to be difficult to make permanent hyperthyroidism by means of TSH administration, whereas the hyperthyroid state is available with TRH administratfion for a long term. The long acting thyroid stimulator (LATS), reported by Adams and Purves,[1] seems to have

a certain role in the development of thyrotoxicosis, but there could not be found any relation between the LATS activity and indices of thyroid function.[19] Although Kriss[15] postulated LATS as a kind of antibody, the mechanism(s) of LATS production remains quite obscure. Kriss said that one could not at that time exclude the possibility that TSH is early implicated in the pathogenetic synthesis of LATS and, via this agent, into hyperthyroidism because in animals, for example, the injection of TSH greatly increases the release of thyroglobulin into the draining lymph[7]: The possibility certainly exists that other proteins of varying degrees of antigenicity may also be released under condition of increased endogenous TSH. Thus intermittent, not continuous, increment of endogenous TSH induces continuous function enhancement of thyroid. In this point of view, our hyperthyroid models induced by a long term administration of TRH will become some beneficial means with a new light to study a pathological physiology of Graves' disease.

SUMMARY

A large dose of synthetic thyrotropin-releasing hormone (TRH), pyroglutamyl histidylproline amide, was administered to rats i. p. once a day for 32 days.

1. High serum PBI levels were observed in the rats treated with 32 days long injection of synthetic TRH, and the high PBI levels continued for additional 36 days after the final administration of TRH.

2. Increases of plasma TSH levels were not detected at 24 hours after the final administration of TRH.

3. Low levels of serum total cholesterol, phospholipids, urea-N, non-protein-N and α_1-globulin were observed in these rats.

ACKNOWLEDGEMENT

The authors are very grateful to Dr. Atsushi Watanabe, President of Daigo Nutritive Chemicals Co. Ltd., for synthesizing and applying thyrotropin-releasing hormone.

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