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Kobe J. Med. Sci. 17, 9-15, March 1971.

NEUROENDOCRINE CONTROL OF THE PITUITARY TSH SECRETION

V. Mode of Action of Synthetic TRF with Special Reference to Interaction between TRF and Thyroxine

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Indexing Words
Thyrotropin-Releasing Factor (TRF); hypothalamus; thyroid hormone

Masahiro SAKODA, Makoto ŌTSUKI, Takaaki KUSAKA, Hidetaro MORI and Takashi MINEYAMA *Neuroendocrine Control of the Pituitary TSH Secretion* V. Mode of Action of Synthetic TRF with Special Reference to Interaction between TRF and Thyroxine. Kobe J. Med. Sci. 17, 9-15, March 1971——Thyrotropin-releasing activity of the tripeptide (pyroglutamyl histidil-proline amide) synthesized by Folkers was observed. Synthetic TRF preparation showed the absence of TSH like activity and did not potentiate TSH. Moreover as we reported previously, there is dose response relationship between the dose of TRF administered and the response of pituitary TSH release, and the effects in vivo and in vitro were the same. From these results we concluded that the activity of synthetic TRF is specific. Synthetic TRF activity was inhibited by thyroxine and the inhibitory effect of thyroxine showed dose dependency between the dose of pretreated thyroxine and pituitary TSH response to the administered TRF.

INTRODUCTION

Thyrotropin (TSH) release from the anterior pituitary gland is dependent on hypothalamic humoral factor which is designated as thyrotropin releasing factor (TRF) and many investigators have attempted to isolate and purify this factor. An intensive chemical research for TRF was carried out by many investigators[2) 11) 13)] before 1969 and it was found that TRF was thought to be characteristic of tripeptide containing histidine, glutamic acid and proline as well as many other structural characteristics. In 1969 Folkers[3)] reported that a synthetic tripeptide preparation, pyroglutamyl-histidyl-proline amide ((Pyro) Glu-His-Pro (NH_2)), had TRF activities. In the course of these procedure, TRF was found to satisfy the criteria as a hormone and called "thyrotropin releasing hormone[12)] (TRH)". It is necessary to examine the specifity of biological action of synthetic TRF to suppose its characteristics as a hormone. We reported on in vivo and in vitro activity of the synthetic TRF supplied by Folkers previously. In this study we attempted to determine the mode (site and mechanism) of action of synthetized TRF and its modifying factors.

Received for publication October 3, 1970
Authors' names in Japanese: 佐古田雅弘, 大槻真, 日下孝明, 森顕太郎, 嶺山隆司

MATERIALS AND METHODS

1. *Experimental animals*

Female mice of D-D strain weighing 13–15g and male rats of Swiss-Webster strain weighing 120–130g were fed on a low iodine diet and distilled water.

2. *Assay procedure of TRF activity*

a) *in vivo* assay on mice

TRF activity was measured after Redding's method[7] with slight modification which employed an increase of mouse blood radioactivity. At zero-time 5μc of Na-^{131}I was injected i.p and two hours later 1.0μg of T_4 was administered s.c. Seventy-two hours after initial injection, blood samples were withdrawn from ophthalmic venous plexus, test samples were injected into tail vein two hours later and the second blood samples were taken from another ophthalmic venous plexus. Radioactivity in corresponding samples of blood taken before and after administration of the test substance was estimated to calculate the increase rate of initial blood ^{131}I at 2 hours after administration.

b) *in vivo* assay on rats

An increase of plasma TSH was employed to demonstrate TRF activity after Sakoda's[9] method; 0.4μg/100gbw of T_4 was injected s.c. 4 hours before experiment. Blood samples were taken from jugular vein 10 minutes after administration of test samples into jugular vein under pentobarbital anesthesia.

c) *in vitro* assay (short term incubation technique after Saffran[8])

The pituitary glands were hemisected and three halves taken from different anterior pituitary were placed into 25ml pyrex beakers containing 2ml of Krebs-Ringer-bicarbonate-glucose-buffer (pH 7. 2). One flask was used for an additional test and the other flask served as control. The incubations were carried out at 37°C in an atmosphere of 95% 0_2 – 5% CO_2. A preincubation of 60 minutes was first carried out and then, on testing the effect of test material (on anterior pituitary), TSH activity released into the incubation medium at final incubation for 60 minites was measured.

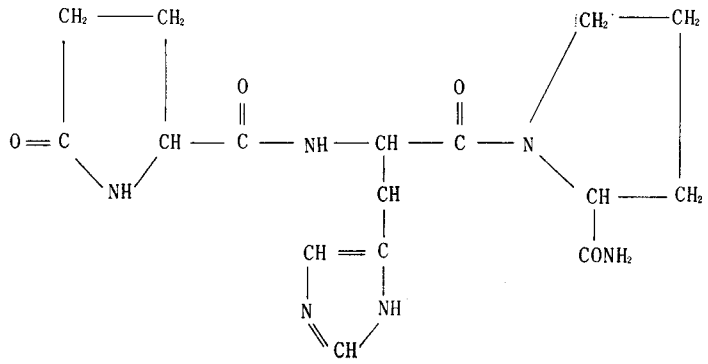
3. *TSH assay*

TSH was measured according to the modified method of McKenzie[6] with precise index of 0.359 and minimal detectable level of 0.1m USP.

4. *TRF preparation*

Synthetic TRF was released by Dr. Folkers[3] (IBR 228, Institute for Biomed. Research, Univ. of Texas), chemical structure of which is shown in Fig. 1, and the powder was dissolved in saline.

Fig. 1 Synthetic thyrotropin releasing hormone (TRH)



L-(pyro) Glu-L-His-L-Pro (NH_2)

5. Hypophysectomy of rat

Hypophysectomy was carried out by transauricular method. Rats were used 72 hours after operation and at the end of experiments, the completeness of hypophysectomy was checked by autopsy.

6. Statistical analysis

Student " t " test was employed for statistical analysis. 8 to 10 mice were used for single determination of TSH.

RESULTS

A. Specificity of action of synthetic TRF

1) Synthetic TRF does not potentiate TSH

To exclude the danger that the synthetic TRF has an potentiating effect of TSH, we directly injected synthetic TRF to mice after incubation of synthetic TRF with bovine TSH (Thytropar, Armour) for 15 minutes at 37°C. The result showed that synthetic TRF did not potentiate TSH. (Table 1)

Table 1. Absence of potentiation for TSH by synthetic TRF

Preparations	^{131}I release from thyroid gland of mice % of initial blood ^{131}I at 2 hrs. ± S. E.	P
bovine TSH* 0.2mUSP	147.0 ± 13.9	——
bovine TSH 0.2mUSP + TRF 10μg	186.0 ± 15.0	N.S.
bovine TSH 0.4mUSP	249.0 ± 26.0	——
bovine TSH 0.4mUSP + TRF 10μg	244.0 ± 19.5	N.S.

* Thytropar (Armour pharmac Co) were used as bovine TSH preparation

2) Synthetic TRF has no TSH-like activities

To exclude the danger that the synthetic TRF has TSH-like activities and acts on the thyroid level, we carried out the following procedures:

(a) Synthetic TRF was directly administered to mice (whose endogeneous TSH secretion was inhibited by pretreatment with 10μg of T_4).

(b) Synthetic TRF was administered to the hypophysectomized rat from which blood samples were collected into heparinezed tube 10 minutes later and plasma TSH was estimated by the slightly modified McKenzie's assay. Both results indicated that synthetic TRF has no TSH-like activities. (Table 2)

Table 2. Absence of TSH activity of synthetic TRF

Preparation	Animal	^{131}I release from thyroid gland % of initial blood ^{131}I at 2 hrs. ± S.E.	P
Saline	Untreated rat	67.0 ± 8.2	——
Synth. TRF 10μg	Untreated rat	324.0 ± 17.1	<0.05
Synth. TRF 5μg	Hypophy-sectomized rat	87.5 ± 4.1	N.S
Synth. TRF 10μg	Hypophy-sectomized rat	88.6 ± 5.4	N.S
Saline	T_4 pretreated mice	80.0 ± 6.0	——
Synth. TRF 0.1μg	T_4 pretreated mice	105.0 ± 7.4	N.S

B. Influence of thyroxine on synthetic TRF activity

Plasma TSH levels after injection of synthetic TRF were estimated in the rats pretreated with various doses of L-thytoxine Na 4 hours before. The rats pretreated with 3.0 and 5.0μg of T_4 showed no TSH increase after administration of 1.0μg synthetic TRF, whereas the rat treated with 3.0 and 5.0μg of T_4 showed distinct

Table 3. Effect of increasing dose of T_4 on synthetic TRF activity

Treatment	^{131}I release from thyroid gland of mice, % of initial blood ^{131}I at 2 hrs. ± S.E.	P
No T_4 + Synth. TRF 1.0μg	236.2 ± 11.0	——
1.0μg T_4 + Synth. TRF 1.0μg	262.2 ± 11.2	N.S
3.0μg T_4 + Synth. TRF 1.0μg	172.2 ± 7.7	<0.005
No T_4 + Synth. TRF 10.0μg	263.0 ± 22.3	——
1.0μg T_4 + Synth. TRF 10.0μg	251.0 ± 20.0	N.S
3.0μg T_4 + Synth. TRF 10.0μg	187.6 ± 4.7	N.S
10.0μg T_4 + Synth. TRF 10.0μg	172.5 ± 12.7	<0.025

TSH increase when 10.0μg of synthetic TRF was administered. However 10.0μg of synthetic TRF could not evoke TSH increase when rats were given 10.0μg of T_4. (Table 3)

It was concluded that there are some doses dependent on negative interaction between synthetic TRF and thyroxine in regulation of pituitary TSH secretion.

DISCUSSION

1. *The specificity of synthetic TRF action*

In the course of isolation and purification of supposed TRF, there is an important problem, that is, to ascertain the specificity of TRF action For this purpose many investigators including us[4) 5) 10)] proposed some criteria for TRF : 1) TRF should act on the pituitary level (i.e. TRF neither activate endegeneous TRF nor act on the thyroid level), 2) TRF should release TSH (i.e. TRF neither potentiates TSH nor inhibits inactivation of TSH), 3) TRF should have dose-response relationship and a certain time pattern of action as characteristics of a hormone. From these points of view it is impotant to investigate the specificity of synthetic TRF though Folkers did not report clearly. We found that synthetic TRF did not potentiate TSH and had no TSH-like activities. Synthetic TRF could evoke TSH release in the rats pretreated with the dose of thyroxine much enough [18)] to inhibit TSH response to cold which is thought to be physiological stimulation for TSH release. These results support the specificity of the action of synthetic TRF. Moreover it showed dose response relationship on which we reported previously, and that the TRF had quite the same activity in vivo and in vitro. From these properties it is concluded that the specificity of synthetic TRF action is established.

2. *Infiuence of thyroxine on synthetic TRF activity*

Vale,[16)] Bowers,[1)] and we[14) 15)] reported that the TSH-releasing activity of purified TRF was inhibited in the rats pretreated with increasing dose of T_4. On the contrary, increasing the dose of TRF overcame the inhibition of T_4.

It is suggested that there was a dose dependent on negative interaction between T_4 and TRF. In this study we investigated the influence of T_4 on synthetic TRF activity and found the same relationship. The rats pretreated with 3.0 and 5.0μg of T_4 showed no TSH increase after administration of 1.0μg synthetic TRF, whereas a distinct TSH increase was observed when 10.0μg of synthetic TRF was administered. However 10.0μg of synthetic TRF could not evoke TSH increase when rats were given 10.0μg of T_4. The pituitary TSH release is regulated by two components i.e. accelerating factor TRF of central nervous origin and inhibitng factor thyroxine of peripheral origin.

CONCLUSION

Using the synthetic TRF ((Pyro) Glu-His-Pro (NH_2)) received from Dr.

Folkers we examined the specificity of action of TRF and interaction between TRF and thyroxine.

1. Synthetic TRF did not potentiate bovine TSH.
2. Synthetic TRF did not show TSH-like activity.
3. There is dose-response relationship between the dose of TRF and response of TSH release, characteristic time response of TSH release. Synthetic TRF has thyrotropin releasing activity in vitro as well as in vivo. From these results we could conclude that the effect of synthetic TRF was specific.
4. T_4 inhibits the effect of synthetic TRF. The inhibitory effect of T_4 is dose dependent between the dose of pretreated T_4 and that of administered synthetic TRF.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Folkers for his kind release of synthetic TRF preparation.

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