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# NEUROENDOCRINE CONTROL OF THE PITUITARY TSH SECRETION

## II. Role and Mode of Pituitary TSH Secretion in the Cold

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*Indexing Words*
**Thyroid stimulating Hormone (TSH);**
**Cold;**
**Thyroid Hormone;**
**Thyrotropin Releasing Factor (TRF)**

Masahiro SAKODA, and Shiro WASIHO. *Neuroendocrine Control of the Pituitary TSH Secretion* II. *Role and Mode of Pituitary TSH Secretion in the Cold.* Kobe J. Med. Sci. 16, 87-94, September 1970——In order to investigate the role and mode of pituitary thyrotropin in the early thyroid response to cold exposure, the contents of TSH in rat plasma and anterior pituitary were determined by means of TSH assay after Mckenzie's modification. Only in the case of rats acclimatized to 28°C a plasma TSH increase was observed by the exposure to 0°C, indicating that the temperature of acclimatization and cold exposure is essential for plasma TSH response. Pituitary TSH contents were decreased coincidentally with the variation of plasma TSH increase, suggesting that the TSH release was evoked by thyrotropin releasing facter (TRF).

The elevation of plasma TSH was inhibited by the pretreatment with thyroid hormone and large dose of atropine, whereas pentobarbitulate did not inhibit the plasma TSH increase.

## INTRODUCTION

There is general agreement upon the fact that the increased secretion of thyroid hormone necessary for thermogenesis during exposure to low environmental temperature depends on the increased secretion of thyrotropin (TSH) from the anterior pituitary. For instance, no increased secretion of thyroid hormone was observed in the animals bearing pituitary stalk section[1] or hypothalamic lesion.[2] Although a good deal of evidence for the increase in TSH secretion on exposure of animals to cold has been presented, the pattern of its discharge is not fully elucidated. In this respect, the author has tried to demonstrate the major role of early thyroid response in the cold, and the contents of TSH in rat plasma and anterior pituitary were determined by means of TSH assay after Mckenzie's modification.

## MATERIALS AND METHODS

Male rats (weighing c. 100 gm., Swiss-Webster strain) fed on a low iodine diet were used.

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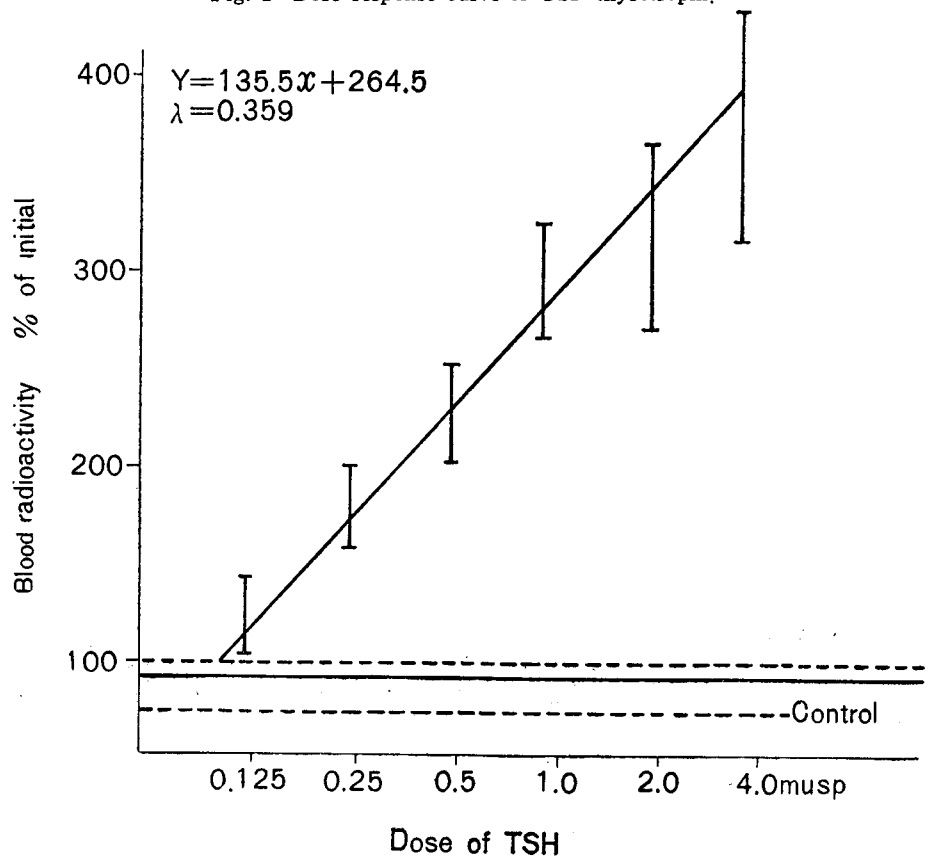
The rats were divided into two groups consisting of 5 rats. One group was kept at room temperature of 29°C for at least 2 weeks before experiment, and the other also at 23°C for 2 weeks. Then these two groups were exposed to cold enviroment of 15°C, 8°C, 0°C and -10°C. Blood samples were obtained with heparinized syringes at every 30 minutes' intervals after cold exposure by the puncture of jugular vein under pentobarbital anaesthesia and immediately centrifuged. The plasma was stored in sterile vials at -20°C until TSH assay. The pituitary glands were collected on ice immediately after decapitation, homogenized with sterile cold saline and centrifuged. These were also stored at -20°C until assay.

Thyroidectomy was performed under pentobarbital anaesthesia 2 weeks prior to the experiment.

Finally, effect of pretreatment with various doses of thyroid hormone ($T_3$ or $T_4$), pentobarbital-Na, and atropin sulfate on the pituitary thyroidal response to cold was investigated.

TSH assay: Each blood sample was assayed by Mckenzie's modification using DD strain (about 15 gm.) of female mouse. The dose response curve to USP thyrotropin is illustrated in Fig. 1.

Fig. 1 Dose response curve to USP thyrotropin.



## RESULTS

Experiment 1. *Effect of cold on rat plasma TSH level*

It was considered of interest to determine whether or not the room temperature to which animals are acclimatized previously may modify the effects of cold on the TSH secretion from the pituitary. Therefore, rats which had been kept under two different thermal conditions such as 29°C or 23°C for at least more than 2 weeks, were exposed to cold of 15°C, 8°C, 0°C and -10°C. In rats acclimatized to room temperature of 29°C, plasma TSH level was significantly increased in 30 minutes ($P<0.01$) after cold exposure to 0°C. On the contrary, rats acclimatized to relatively low room temperature of 23°C showed a similar but a little prolonged elevation of the plasma TSH levels in 30 minutes exposure to 0°C, but this response was not significant. When rats were exposed to a severe cold of -10°C or moderate cold of 15°C, 8°C, there was no increment of the plasma TSH content in 30 minutes (Table 1).

Table 1 Effect of cold exposure in rat plasma TSH level

(Exposure 30 min.)

| Treatment | $I^{131}$ release from thyroid gl. of mice % of initial blood $I^{131}$ at 2 hr.±S. E. | P |
|---|---|---|
| Non-exposure | 95.2± 4.3 | |
| 23±1°C→ 15°C | 138.0±13.9 | N.S. |
| 23±1°C→ 8°C | 140.1±35.5 | N.S. |
| 23±1°C→ 0°C | 108.4± 8.1 | N.S. |
| 23±1°C→-10°C | 79.9±15.9 | N.S. |
| 29±1°C→ 0°C | 226.4±24.1 | <0.01 |
| 29±1°C→-10°C | 140.7±24.4 | N.S. |

Experiment 2. *Effect of cold on rat plasma and pituitary TSH level*

When rats were exposed to from 29°C to 0°C for 6 hours, the plasma TSH levels were increased markedly only in 30 minutes exposure and the peak was reached 1 hour after cooled ($P<0.01$), then this high TSH level remained as it was as long as 3 hours. ($P<0.01$), (Table 2).

Table 2 Changes in plasma TSH level after cold exposure

(29°C±1°C→0°C)

| Treatment | $I^{131}$ release from thyroid gl. of mice % of initial blood $I^{131}$ at 2 hr.± S.E. | P |
|---|---|---|
| Non-exposure | 83.1±11.4 | |
| 30 min. expos. | 243.0± 6.9 | <0.01 |
| 1 hr. expos. | 401.7±22.0 | <0.01 |
| 3 hrs. expos. | 213.5±15.7 | <0.01 |
| 6 hrs. expos. | 178.7±24.4 | N.S. |

As shown in Table 3, during the initial stages of cold exposure at 0°C, pituitary content of TSH decreased in 30 minutes ($P<0.05$) and in 1 hour ($P<0.05$). Then they returned to a control level in 3 hours.

Table 3 Changes in pituitary TSH contents after cold exposure.
(29°C±1°C→0°C)

| Treatment | $I^{131}$ release from thyroid gl. of mice % of initial blood $I^{131}$ at 2 hr± S.E. | P |
|---|---|---|
| Non-exposure | 228.2±20.0 | |
| 30 min. expos. | 109.2± 6.2 | <0.05 |
| 1 hr. expos. | 109.7±15.0 | <0.05 |
| 3 hrs. expos. | 295.0±20.6 | N.S. |
| 6 hrs. expos. | 206.4±22.3 | N.S. |

Experiment 3. *Factors affecting rat plasma TSH level in the cold*

Then, next, factors which affect increment of rat plasma TSH level in 30 minutes cold exposure (29°C→0°C) were studied.

i) Effect of thyroid hormone

When rats treated with 3-5-3′ l-Tri-iodethyronine ($T_3$) for 3 days and then another injection at 4 hours prior to experiment on the 4th day, there was no increment in rat plasma TSH level (Table 4).

Table 5 shows effect of pretreatment with l-thyroxine Na ($T_4$) 1—30 μg 4 hours prior to experiment. From 10 μg to 30 μg dosis of $T_4$ suppressed TSH release from the pituitary significantly. Smaller dosis of $T_4$ (1—5 μg) showed a similar but a slight suppression of the plasma TSH level, but not significant.

Table 4 Changes in plasma TSH level after cold exposure under pretreatment with $T_3$. (29°C±1°→0°C, 30 min.)

| Treatment | $I^{131}$ release from thyroid gl. of mice % of initial blood $I^{131}$ at 2 hr.±S.E. | P |
|---|---|---|
| $T_3$ 10 μg/100 g B.W./day for 3 days | 86.0± 4.6 | |
| $T_3$ 10 μg/100 g B.W./day for 3 days and cold expos. | 101.2±11.1 | N.S. |
| $T_3$ 10 μg/100 g B.W. | 108.0±19.0 | |
| $T_3$ 10 μg/100 g B.W. | 146.8±17.0 | N.S. |

Table 5 Effect of various dosis of $T_4$ in rat plasma TSH level after cold exposure. (29°C±1°C→0°C)

| Treatment | $I^{131}$ release from thyroid gl. of mice % of initial blood $I^{131}$ at 2 hr.±S. E. | P |
|---|---|---|
| $T_4$ 1 μg 4 hrs. before cold exposure | 148.1±13.6 | N.S. |
| $T_4$ 3 μg 4 hrs. before cold exposure | 131.1±11.7 | N.S. |
| $T_4$ 5 μg 4 hrs. before cold exposure | 118.5±10.6 | <0.05 |
| $T_4$ 10 μg 4 hrs. before cold exposure | 94.4±16.0 | <0.01 |
| $T_4$ 30 μg 4 hrs. before cold exposure | 96.5±13.2 | <0.05 |
| $T_4$ untreated | 226.4±24.1 | |

ii) Effect of thyroidectomy

Thyroidectomized animals showing high plasma TSH levels 2 weeks after operation were exposed to cold at 0°C for 30 minutes. An increase of plasma TSH levels was observed also in this case. It is suggested that TSH response of thyroidectomized animals to cold was rather similar to normal acclimatized animals. When these rats were pretreated with $T_4$ (5 μg—10 μg/day) on the 11th, 12th and 13th day after operation, lower plasma TSH levels than the $T_4$ untreated thyroidectomized animal's were observed. Then these animals were exposed to cold (29°C→0°C), and no increment of the plasma TSH content was observed (Table 6).

Table 6 Effect of Thyroxine on plasma TSH level of thyroidectomized rat after cold exposure. (29°±1°C→0°C, 30 min.)

| Treatment | $I^{131}$ release from thyroid gl. of mice % of initial blood $I^{131}$ at 2 hr.±S. E. |
|---|---|
| Thyroidex.**+$T_4$ 5 μg for 3 days*** and exposure | 113.7±29.1 |
| Thyroidex.**+$T_4$ 10 μg for 3 days*** and exposure | 117.3±24.3 |
| Thyroidex.**+exposure $T_4$ untreated | 427.7±16.1* |
| Thyroidex.**+non-exposure | 227.0±15.5* |

* 0.2 ml. plasma

** thyroidex. 2 weeks previously

*** given on the 11th, 12th and 13th post-operative day

iii) Effect of pentobarbital-Na, or atropin sulfate

Pretreatment with pentobarbital-Na-4.5 mg/100 gm B.W. or atropin sulfate 30 μg/100gm B. W., 175 μg/100 gm B. W. did not prevent TSH response to cold, and only larger dosis of atropin sulfate (350 μg/100 gm) prevented TSH secretion from the pituitary (Table 7).

Table 7 Changes in plasma TSH level after cold exposure under pretreatment with pentobarbiturate or atropin.

(29°C±1°C→0°C, 30 min.)

| Treatment* | $I^{131}$ release from thyroid gl. of mice % of initial blood $I^{131}$ at 2 hr.±S. H. | P |
|---|---|---|
| Untreated | 243.0±35.0 | |
| Pentobarbital-Na 4.5 mg/100 g B.W. | 199.9±10.4 | N.S. |
| Atropin sulfate 30 μg/100 g B.W. | 181.8±28.1 | N.S. |
| Atropin sulfate 175 μg/100 g B.W. | 198.0±43.6 | N.S. |
| Atropin sulfate 350 μg/100 g B.W. | 108.0±10.7 | <0.01 |

* Treatment took place 15 min. prior to cold exposure.

## DISCUSSION

The modern concept of pituitary thyroid function on cold exposure postulates that intact hypothalamo-pituitary connections are necessary for the maintenance of physiological response.[1) 2) 3) 4)]

Furthermore, it was reported, this response was altered by treatment with thyroid hormone especially in chronic cold exposure. In this case the pituitary response to cold exposure seems to be still obscure including neural or feed back moment.

We have investigated the response of plasma TSH of rat acclimatized to 28°C and 23°C in early stages of exposure to 15°C, 8°C, 0°C, and -10°C. Only the case of rats acclimatized to 28°C showed increment of plasma TSH by the exposure to 0°C and this increase has lasted for 6 hours. On the contrary, in the group acclimatized to 23°C there could not be observed any increase of plasma TSH during 1 hour cold exposure. Only in case of sustained exposure for 3 hours, plasma TSH was increased. These findings point out the essential role of plasma TSH in thyroid response after cold exposure.

Also the temperature of acclimatization and cold exposure is essential for plasma TSH response.[4)] In the early stages of cold exposure, plasma TSH increment was accompanied with a decrease of pituitary TSH contents. This means

the TSH release is essential for pituitary thyroid response after cold exposure indicating the role of hypothalamic hormone TRF. Sakoda and Nakabayashi studied the fluctuation of rat hypothalamic TRF and explored the increased TRF activity in the rat hypothalamus exposed to cold.

Rats treated with 5 $\mu$g or more of $T_4$ showed no plasma TSH increase after 30 min cold exposure. Pretreatment with 1 and 3 $\mu$g of $T_4$ also diminished pituitary TSH response to cold.

There is no observation on effect of thyroid hormone upon the pituitary TSH response after cold exposure although Yamada[4] reported on the inhibitory effect of thyroid hormone on the response of thyroid gland after cold exposure.

Although their plasma TSH levels were already high, thyroidectomized group of rats showed distinct plasma TSH increase by 30 min cold exposure. Plasma TSH response to cold was completely inhibited in thyroidectomized rat treated with 5 or 10 $\mu$g of $T_4$ given daily on the 11th, 12th and 13th post-operative day.

From these results, pituitary TSH response was facilitated by hypothalamic TRF and was inhibited by the circulatory thyroid hormone at the early stages of cold exposure.

As shown in Guillemin's[5,6] and Bowers'[7,8] early reports, it may be considered that TRF activity is highly dependent on circulatory thyroid hormone level. So there is so-called "pituitary selective response" in the cold exposure.

Pretreatment with pentobarbitulate and atropine did not inhibit the TSH increase after cold, although a relative larger dose of atropine prevented the TSH response. Further study will have to be made as regards time and dosis of atropine administration.

## SUMMARY

1. Plasma TSH increase was observed in the rat acclimatized at 28°C and then exposed to 0°C.

2. Rat acclimatized to 23°C showed no TSH increase even after exposure to 0°C.

3. Mild cold (15°C, 8°C) or severe cold (−10°C) did not cause plasma TSH increase.

4. These plasma TSH increases were accompanied with a decrease of pituitary TSH contents.

5. Thyroidectomized rat acclimatized at 28°C showed plasma TSH increase after exposure to 0°C.

6. Pretreatment with thyroid hormone or larger dosis of atropine inhibited plasma TSH response after cold exposure.

## REFERENCES

1. UOTILA, U. U.
Endocrinology 1939. **25**. 605/614
On the Role of the Pituitary Stalk in the Regulation of the Anterior Pituitary, with Special Reference to the Thyrotropic Hormone.

2. KNIGGE, K. M., and BIERMAN, S. M.
Am. J. physiol. 1958 **192**. 625/1958
Evidence of Central Nervous System Influence upon Cold-induced Acceleration of Thyroidal $I^{131}$ Release.

3. D'ANGELO, S. A.
Am. J. physiol. 1960. **199**. 701/706
Hypothalamus and endocrini function in persistent rats at low enviromental temperature.

4. YAMADA, T., KAJIHARA, A., ONAYA, T., KOBAYASHI, I., TAKEMURA, Y., and SHICHIJO, K.
Endocrinology 1965. **77**. 968/976
Studies on acute stimulatory effect of cold on thyroid activity and its mechanism in the guinea pig.

5. GUILLEMIN, R., E. YAMAZAKI, D. A. GARD, M. JUTISZ, and SAKIZ, E.
Ibid 1963. **73**. 564/572
*In vitro* secretion of thyrotropin (TSH) : Stimulation by a hypothalamic peptide (TRF).

6. VALE, W., BURGUS, R., and GUILLEMIN, R.
Proc. Soc. Exp. Biol. Med. 1967. **125**. 210/213
Competition between thyroxine and TRF at the pituitary level in the release of TSH.

7. BOWERS, C. Y., SCHALLY, A. V., REYNOLDS, G. A., and HAWLEY, D. W.
Endocrinology 1967. **81**. 741/747
Interaction of l-Thyroxine or l-Triiodothyronine and Thyrotropin-Releasing Factor on the release and Synthesis of Thyrotropin from the anterior pituitary gland of mice.

8. BOWERS, C. Y., LEE, K. L., and SCHALLY, A. V.
Ibid 1968. **82**. 75/82
A study on the interaction of the Thyrotropin-Releasing Factor and l-Triiodothyronine : Effect of puromycin and cycloheximide.