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Studies on the Thyrotropin-Releasing Hormone (TRH)* Activity in Peripheral Blood† (33892)

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There is overwhelming evidence to support the concept that neurohormones secreted into the hypophyseal portal vessels control the release of anterior pituitary hormones (1–3). However, there is little data available on the presence of these neurohormones in the hypophyseal portal blood or in peripheral blood. Recently, Fink *et al.* (4) detected LRF in hypophyseal portal blood using a technique devised by Worthington (5). It seemed worthwhile to investigate TRH activity in the peripheral blood of rats under certain experimental conditions. The detection of this hormone in peripheral circulation has been difficult because of the relatively low sensitivity of the presently available assay systems. Furthermore, our laboratory demonstrated that blood rapidly inactivates TRH (6). Nevertheless, by subjecting the animals to certain physiological manipulations and by increasing the sensitivity of the TRH assay, we demonstrated a TRH-like activity in peripheral blood in response to the stimulus of mild cold. A study was also made of the inactivation of TRH in rats subjected to these various treatments in an attempt to shed some light on the conditions under which TRH can be detected in the peripheral circulation.

Materials and Methods. Female Sprague-Dawley weanling rats, weighing 75–90 g, were subjected to hypophysectomy to eliminate any effects due to endogenous TSH. After 30 days some rats were subjected to surgical thyroidectomy. Five days later they were separated into two groups of 5 to 6 animals. The experimental group was then exposed to cold at 5° for 2 hr while the control animals were maintained at room temperature. In an-

* The terminology proposed by Schally *et al.* (1) will be used in this paper.

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TABLE I. Effect of Cold Exposure on Plasma TRH-Like Activity in Hypophysectomized Rats.[a]

Plasma source	^{125}I release from thyroid gland; change in ^{125}I levels in blood of mice at 2 hr (Δcpm $\pm$ SE)	p[b]
Saline[c]	-20 ± 10	—
Controls hypophysectomy 30 days	-14 ± 10	—
Hypophysectomy 30 days + cold exposure	-11 ± 12	NS
Saline[c]	21 ± 12	—
Controls hypophysectomy 30 days	70 ± 26	—
Hypophysectomy 30 days + cold exposure	15 ± 8	NS

[a] Four–5 rats/group were used.

[b] The differences between the average responses (cpm) of control and the cold exposed rats were compared.

[c] Response to saline in bioassay mice.

other experiment the rats subjected only to hypophysectomy were treated in the same manner as described above. All groups were anesthetized with ether and heparinized blood was quickly removed from the abdominal aorta. Blood from rats having pituitary fragments upon autopsy was excluded. Pooled blood samples from 4 to 5 rats were quickly chilled on ice and the plasma was separated by centrifugation at 2°. The plasma was then immediately assayed for TRH.

TRH Assay. The *in vivo* TRH assay is based upon the concept that when TRH is injected into an intact mouse, a release of TSH from the anterior pituitary occurs. This in turn increases the rate of release of labeled thyroid hormone from the thyroid gland and the response is measured as a change in blood radioactivity. Female, white Swiss mice, weighing 15–20 g were maintained on a low iodine diet for 3–5 weeks. On the day before the assay, they were injected intraperitoneally with 5 μCi of sodium iodide ^{125}I along with 0.085 μg of triiodothyronine (T3) dissolved in 0.01 *N* NaOH: 1% albumin solution. Twenty-four hr later the animals were ready for use in the assay. Blood samples were withdrawn from the retro-orbital sinus of the eye into 55-μl heparinized capillary tubes. Plasma, or other materials, were injected in a volume of 0.2 ml into the tail vein. Five to six mice were used per group. Two hr later a second blood sample was withdrawn and the radioactivity in corresponding samples was counted to a 2% probable error in a scintillation spectrometer. To ascertain whether TSH was present, some samples were assayed for TSH by the method of McKenzie (7). Statistical analyses were performed as reported previously (8).

In vitro *inactivation studies.* To study the inactivation of TRH *in vitro* in plasma, a known quantity of highly purified TRH of porcine origin (1, 9) was added to a 1:8 dilution of plasma in Krebs–Ringer bicarbonate medium, pH 7.4 (10). After an incubation period of 30 min at 37°, the solution was quickly chilled on ice and assayed immediately for TRH. TRH prepared at the same concentration, but without the plasma served as a control.

Results. Table I shows the results obtained with rats hypophysectomized 30 days before exposure to cold. Plasma from these rats did not show TSH or TRH-like activity. Consequently, it was concluded that rats subjected to hypophysectomy and exposure to cold do not have demonstrable TRH activity in peripheral blood. We then subjected another group of rats to thyroidectomy in addition to hypophysectomy. Table II indicates that in 3 experiments, plasma from such rats exposed to cold had a significant TRH-like activity. The mean difference in plasma TRH-like activity between the control hypohysectomized–thyroidectomized rats and experimental rats subjected to mild cold was highly significant. Absence of a response when the same plasma samples were assayed for TSH indicates that the responses seen in the TRH assay were not due to TSH. Therefore, it would appear that the responses in our assay were due to endogenous TRH or TRH-like substances found in blood of hypophysectomized–thyroidectomized rats after the exposure to cold.

The effect of plasma from rats subjected to various treatments on inactivation of TRH

TABLE II. Effect of Cold Exposure on Plasma TRH-Like Activity in Hypophysectomized-Thyroidectomized Rats.[a]

Plasma source	^{125}I release from thyroid gland; change in ^{125}I levels in blood of mice at 2 hr (Δcpm ± SE)	p^b
Saline[c]	−17 ± 1	—
Control hypox 30 days and thyrx 5 days	−16 ± 28	—
Hypox 30 days and thyrx 5 days after cold exposure	173 ± 53	.01
Saline[c]	−79 ± 23	—
Control hypox 30 days and thyrx 5 days	−292 ± 283	—
Hypox 30 days and thyrx 5 days after cold exposure	660 ± 243	.01
Saline[c]	−260 ± 197	—
Control hypox 30 days and thyrx 5 days	−47 ± 12	—
Hypox 30 days and thyrx 5 days after cold exposure	85 ± 19	.01

[a] Four–5 rats/group were used.

[b] The differences between the average responses (cpm) of control and the cold exposed rats were compared.

[c] Response to saline in bioassay mice.

in vitro is shown in Table III. Experiment 1 shows that plasma from hypophysectomized rats, diluted 1:8, caused a 29% inactivation of the added TRH in 30 min. Normal rat plasma at the same dilution induced a 83–87% inactivation of TRH. However, as shown in Expts. 1 and 2, plasma from hypophysectomized rats, that had been thyroidectomized 10 days previously had no TRH-inactivating activity, while plasma from rats thyroidectomized for 3 days, inactivated 57% of the TRH. When thyroidectomized–hypophysectomized rats were pretreated with 10 μg of triiodothyronine subcutaneously for 3 days, there was a rapid increase in the ability of the plasma to inactivate TRH.

Table IV reports the inactivation of TRH by individual plasma samples from normal, hypophysectomized, thyroidectomized–hypophysectomized, or triiodothyronine-treated, hypophysectomized–thyroidectomized rats. Again, inactivation of TRH was greater in normal and hypophysectomized–thyroidectomized rats receiving triiodothyronine and smaller in untreated hypophysectomized–thyroidectomized rats.

Figure 1 illustrates the relatively rapid increase of TRH-inactivating enzyme in the plasma of hypophysectomized–thyroidectomized rats 24 hr after a single intravenous injection of 20 μg of triiodothyronine (T3). This demonstrates the stimulating effect of T3 on the enzyme system responsible for inactivation of TRH. There appears to be a 2–3-fold increase in TRH-inactivating activity within 24 hr after the injection of T3.

Discussion. Several studies have shown that mild cold will increase thyroid activity 11–13). It was postulated that the mild cold stimulus, acting through a neuroen-

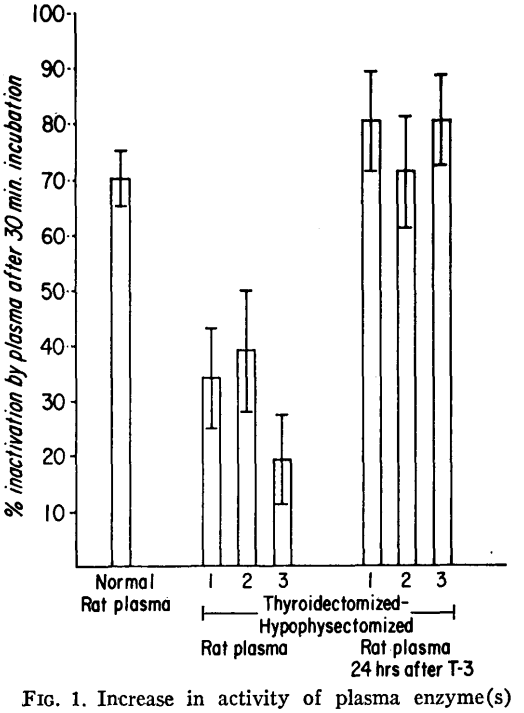

FIG. 1. Increase in activity of plasma enzyme(s) which destroys TRH, 24 hr after a single injection of triiodothyronine (20 μg iv) into hypophysectomized–thyroidectomized rats.

TABLE III. *In Vitro* Inactivation of Thyrotropin-Releasing Hormone by Plasma from Rats Subjected to Various Treatments.

Sample	^{125}I release from thyroid gland; change in ^{125}I levels in blood of mice at 2 hr (Δcpm ± SE)	Inactivation (%)	p^a
Expt. 1			
Standard TRF	4136 ± 861	—	—
Normal rat plasma	681 ± 165	83	—
Plasma from rats hypophysectomized 30 days	2921 ± 224	29	.01
Plasma[b] from rats hypophysectomized 30 days and thyroidectomized for 10 days	4201 ± 965	0	.01
Plasma[b] from hypophysectomized 30 days, thyroidectomized 5 days, T3-treated rat	650 ± 92	84	NS
Expt. 2			
Standard TRH	2766 ± 362	—	—
Normal rat plasma[b]	351 ± 149	87	—
Plasma from rats hypophysectomized 30 days and thyroidectomized for 3 days	1188 ± 78	57	.01

[a] The differences between the average responses (cpm) of normal rat plasma and plasma from experimental groups were compared.

[b] Diluted 1:8.

docrine reflex, may enhance the release of TRH and thus evoke a secretion of TSH from the anterior pituitary (14). The importance of the hypothalamus has been emphasized by several investigators and it has been shown that the neural stimulus to TSH secretion in acute cold is blocked by prior administration of thyroxine or by transplantation of the anterior pituitary from the pituitary fossa to a distant site (14). In our studies we used a sensitive assay able to detect very small amounts of TRH activity. Since the rats were subjected to hypophysectomy, contamination with TSH could not complicate interpretation of our results and it can be assumed with reasonable certainty that we were indeed dealing with TRH-like activity.

TABLE IV. Inactivation of TRH by Plasma from Rats Subjected to Various Treatments.

Sample and treatment	^{125}I release from thyroid gland; change in ^{125}I levels in blood of mice at 2 hr (Δcpm ± SE)	Inactivation (%)	p^a
Standard TRH	1478 ± 190	—	—
Normal rat plasma	491 ± 53	66	—
Plasma[b] from rats hypophysectomized for 30 days	818 ± 97	45	.025
	808 ± 19	45	.001
	975 ± 204	34	.05
Plasma[b] from rats hypophysectomized for 30 days and thyroidectomized for 5 days	1070 ± 130	28	.005
	1116 ± 115	24	.001
	1093 ± 271	26	NS
Plasma[b] from rats hypophysectomized for 30 days and thyroidectomized for 5 days treated for 3 days with triiodothyronine	631 ± 89	57	NS
	64 ± 214	96	NS
	−20 ± 65	100	NS

[a] The differences between the average response (cpm) of normal rat plasma and plasma from experimental groups were compared.

[b] Individual plasma samples.

In our experiments, rats subjected to hypophysectomy alone did not appear to release detectable amounts of TRH after exposure to mild cold. This was surprising, since various investigators detected other hypothalamic releasing hormones in the peripheral blood of hypophysectomized rats (1, 15, 16).

Sinha and Meites (17) reported that hypothyroid rats contained more TRH in the hypothalamus than normal rats and that the hypothalamic TRH content of intact rats was unaffected by the administration of thyroxine. From our data it appears that thyroidectomized–hypophysectomized rats exposed to mild cold release detectable amounts of TRH. This may be due to the fact that thyroidectomy induced greater storage of TRH in the hypothalamus and also caused partial disappearance of the enzyme which destroys TRH. Under these conditions it was possible to obtain detectable release of TRH in the peripheral blood in response to mild cold. Thus mild cold is capable of acting as a specific stimulus for the release of TRH in rats. This provides the first evidence for one of the possible physiological roles of TRH. The absence of a response when these plasma samples were assayed by the McKenzie method (7) indicates that the response in our assay system was due to endogenous TRH or TRH-like substance. Absolute confirmation of the presence of TRH in the peripheral circulation will have to await chemical means for identification of picogram or nanogram amounts of this substance.

Our previous work on inactivation of TRH by a blood enzyme (6, 18), and the fact that after thyroidectomy combined with hypophysectomy and mild cold we could detect a TRH-like activity in plasma, prompted us to investigate further the effect of thyroid hormones on this blood enzyme. When plasma from thyroidectomized–hypophysectomized rats and normal rats were compared, plasma of rats subjected to thyroidectomy in combination with hypophysectomy inactivated less TRH. Thus hypophysectomy alone was not sufficient in reducing thyroid hormone levels to a degree whereby this TRH-inactivating enzyme could no longer operate.

When hypophysectomy was combined with thyroidectomy we detected TRH in the peripheral blood after exposing the animals to mild cold. This seemed to be correlated to some degree with the disappearance of the inactivating enzyme in the plasma. When thyroid hormone was injected into the animals, TRH inactivating enzyme reappeared in the plasma and could be detected within 24 hr after a single injection of triiodothyronine. The reappearance of the enzyme is thus much more rapid than the period required for its disappearance. The exact mechanism by which the thyroid hormone raises the ability of plasma to inactivate TRH is not clear. It is known, however, that hormones may influence the rate at which new enzyme molecules are produced and may act by converting inactive enzymes to an active form (19).

Thus the thyroid hormones may not only regulate TSH secretion by blocking the effect of TRH on the pituitary (20, 21), but may also modify TRH levels in the blood by regulating the enzyme responsible for TRH destruction. Our studies on the destruction of TRH by plasma would seem to indicate that the thyroid hormone may play a role in regulating the level of TRH-inactivating enzyme in the peripheral blood. It would be of interest to determine the effect of various physiologic conditions and drugs used in various thyroid disorders, on the level of this enzyme in the blood.

Summary. Significant TRH-like activity was detected in the blood of thyroidectomized–hypophysectomized rats after (but not before) exposure to mild cold. Thus mild cold is capable of acting as a specific stimulus for the release of TRH in rats. This provides the first evidence for the possible physiologic importance of TRH. A study was made of the conditions under which TRH can be detected in blood. The presence of TRH in the peripheral circulation may be dependent upon the levels of the enzyme(s) responsible for its destruction. TRH is not detectable in the peripheral circulation unless the endogenous level of thyroid hormones is sufficiently reduced. Thyroid hormones are able to influence the levels or activity of the

enzyme(s) responsible for the inactivation of TRH.

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