FEEDBACK MECHANISM BETWEEN THYROID HORMONES AND HYPOTHALAMUS*

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It is a fact of great interest that when ^{131}I -thyroxine and ^{131}I -triiodothyronine are exogenously given, they are highly concentrated in the anterior hypothalamus, median eminence, and hypophysis.

Schittenhelm and Eisler (1932) already found that the intravenous injection of 1.0 mg/kg of thyroxine (T_4) into dogs, cats and rabbits elevated iodine concentration in the tuber cinereum 10 times.

Since the introduction of ^{131}I - T_4 , studies in this field have made a remarkable advance, and Harper and Mattis (1950, 1951), Jensen and Clark (1951), and Courrier *et al.* (1951), using this method, nearly simultaneously confirmed the results of Schittenhelm and Eisler (1932). Although this phenomenon was observed very distinctly in dogs and rabbits, it did not seem to occur so remarkably in all the animals. ^{131}I - T_4 concentration was found in the descending order, in the neurohypophysis, the stalk, and the median eminence. Ford *et al.* (1955) obtained a similar result with ^{131}I - T_3 , and Courrier (1957) reported that 3,5',3'-L- T_3 showed the highest affinity to these sites, followed by 3,3'-L-diiodothyronine and T_4 . Taurog *et al.* (1956,1957) could not observe such accumulation of thyroid hormone in rats and guinea pigs. According to Ford *et al.* (1957), there is difference between ^{131}I - T_4 and ^{131}I - T_3 in the site of concentration, the latter being concentrated in the site where tissue oxygen consumption is higher. In experiments with rabbits, Ford and Gross (1958a) confirmed that T_3 was principally concentrated in Nucleus paraventricularis and the median eminence, and especially highly in the neurohypophysis. Ford and Gross (1958b) further observed the concentration of T_3 in the neurosecretory system in guinea pigs. These reports suggest that T_3 and T_4 are related, at a higher level than the hypophysis, to the hypothalamo-hypophysio-thyroidal system. Previously the authors extracted thyrotrophin releasing factor (TRF) from the anterior hypothalamus, including neurosecretory nuclei, which controlled the hypophysio-thyroidal system, and was presumably related to exogenous T_3 and T_4 . The relationship between the secretion of TSH and the concentration of T_3 or T_4 in the hypothalamus was therefore studied by the administration of non-labelled T_3 and $-\text{T}_4$.

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MATERIALS AND METHODS

I. Materials

Adult male dogs, weighing 10~15 kg were used. Female animals were not used because of the possible influence of sexual cycle. Through the course of experiments, special care was taken to keep the room temperature, light and nutrition as constant as possible.

II. Methods(1) Determination of T_3 and T_4 in the hypothalamus and hypophysis

Two hrs. after intramuscular injection of T_3 and T_4 , animals were sacrificed by electroshock, and the hypophysis and hypothalamus were excised. The hypothalamus was fixed with acetone, frozen with dry ice and divided into anterior, medial and posterior parts to be weighed separately. The anterior part included the optic chiasm and the frontal rim of the median eminence, the posterior part covered the area from the dorsal rim of the median eminence to the mamillary body, and the medial part was the area between the above two. The hypophysis was not divided into the anterior and posterior lobe, to keep the error of the measurement as small as possible. The 3 parts of the hypothalamus and the hypophysis were homogenized, subjected to enzymic hydrolysis by the Suzuki's method (1956), adjusted to pH 5, and extracted 3 times with 10, 5, and 5 volumes of n-butanol, respectively. These extract solutions were desiccated and solidified at temperature below 45°C under a decreased pressure, and then dissolved in 0.4 ml of 30% isopropanol for extraction. After 10 mins. centrifugation, 0.1 ml of the supernatant was placed on Toyo Roshi filter paper No. 50 for quantitative analysis. Blood concentrations of T_3 and T_4 were determined by paper partition chromatography according to the Tomie's method (1959). These were expressed in term of iodine contained in the spots of T_3 and T_4 .

(2) Determination of blood TRF was performed by the Yamamoto's method (1959). Unit of TRF was showed as the amount of TSH, which was determined according to Crooke and Mathews (1953) and expressed in term of J.S.U. (Shibusawa *et al.*, 1959).

(3) Determination of PBI was performed according to the method of Barker *et al.* (1950).

(4) Determination of blood T_3 and T_4 was performed, as afore mentioned, by the method of Tomie (1959).

(5) T_3 and T_4 are commercial products of Sigma Co. They were dissolved in distilled water adjusted to pH 8.5, and 5~7 $\mu\text{g/kg}$ weight was intramuscularly injected at one time.

RESULTS

(1) Concentrations of T_3 and T_4 in the hypothalamus and hypophysis after the intramuscular administration of T_3

As shown in Table 1, the content of T_4 per each 1 mg of the posterior hypothalamic tissue was perfectly same with the control. T_3 concentration also scarcely differed from the control, though in some cases it was impossible to determine the concentration. No significant difference from the control was observed in the concentrations of T_4 and T_3 in the medial hypothalamus, though in some cases the determination of T_3 concentration was impossible. In the anterior hypothalamus, T_4 concentration increased after the injection of T_3 , but there was no significant difference in T_3 from the control. In the hypophysis, scarcely any difference from the control was observed in T_4 concentration, while T_3 was significantly increased. Levels of PBI, T_3 , T_4 , and TRF in the peripheral venous blood showed, as exhibited in Table 2, evident decrease at 2 hrs. after the injection. It is of significant that the level of TRF showed a decrease following the intramuscular injection of T_3 .

Table 1. Concentration of T_3 and T_4 in the hypothalamus and hypophysis of dogs after the intramuscular injection of T_3

	No. of dogs	Wet tissue weight (mg)*	T ₄ concentration		T ₃ concentration		
			(μg)*	(μg/mg tissue weight)*	(μg)*	(μg/mg tissue weight)*	
Posterior hypothalamus							
T ₃ injected	8	883±13	0.044±0.013	0.50 ±0.14×10 ⁻⁴	0.005±0.003	0.05 ±0.03×10 ⁻⁴	
Control	5	785±21	0.039±0.008	0.51 ±0.12×10 ⁻⁴	0.003±0.002	0.04 ±0.02×10 ⁻⁴	
Medial hypothalamus							
T ₃ injected	7	853±62	0.062±0.009	0.72 ±0.13×10 ⁻⁴	0.005±0.004	0.05 ±0.03×10 ⁻⁴	
Control	5	832±38	0.059±0.003	0.71 ±0.24×10 ⁻⁴	0.004±0.002	0.05 ±0.02×10 ⁻⁴	
Anterior hypothalamus							
T ₃ injected	9	635±67	0.116±0.029	1.62 ¹ ±0.43×10 ⁻⁴	0.065±0.005	1.02 ±0.09×10 ⁻⁴	
Control	6	582±84	0.063±0.002	1.08 ¹ ±0.35×10 ⁻⁴	0.062±0.004	1.06 ±0.07×10 ⁻⁴	
Hypophysis							
T ₃ injected	6	137± 9	0.43 ±0.010	3.15 ±0.74×10 ⁻⁴	0.027±0.005	1.96 ² ±0.35×10 ⁻⁴	
Control	5	132±11	0.044±0.009	3.34 ±0.68×10 ⁻⁴	0.016±0.006	1.21 ² ±0.43×10 ⁻⁴	

* Mean $\pm$ Standard error

The differences between the pairs values 1 and 2 groups in Students t-test; 1: $P < 0.05$, 2: $P < 0.05$

Table 2. Concentration of PBI, T_3 , T_4 , and TRF in the peripheral venous blood of dogs after the injection of T_3

	No. of dogs	PBI (μ g%)*	T_4 (μ g%)*	T_3 (μ g%)*	TRF† (TSH, J.S.U.%)*
Before injection	6	2.5 $\pm$ 0.3	2.0 $\pm$ 0.2	0.3 $\pm$ 0.1	0.25 $\pm$ 0.01
2 hrs. after injection	8	1.8 $\pm$ 0.4	1.0 $\pm$ 0.1	0.2 $\pm$ 0.1	0.13 $\pm$ 0.005

* Mean $\pm$ Standard error

† TRF value was showed as equivalent potency of TSH (J.S.U.). See text.

It was thus demonstrated that the administration of T_3 into the systemic circulation increased the concentration of T_4 in the anterior hypothalamus, and that of T_3 in the hypophysis, while no changes in the concentrations of T_3 or T_4 were seen in the medial and posterior hypothalamus.

(2) *Concentrations of T_3 and T_4 in the hypothalamus and hypophysis after the intramuscular administration of T_4*

In the posterior and medial hypothalamus, T_3 and T_4 concentrations did not show any significant difference from the control (Table 3). In the anterior hypothalamus, however, the level of T_4 showed any increase over the control, and that of T_3 also a marked increase. In the hypophysis, the level of T_4 increased over the control.

PBI, T_3 , T_4 , and TRF in the peripheral venous blood all showed a decrease at 2 hrs. after the injection of T_4 (Table 4). It was thus shown that exogenously given T_4 increased the concentrations of T_3 and T_4 in the anterior hypothalamus, whereas the medial and posterior hypothalamus did not respond to this administration.

Table 3. Concentrations of T_3 and T_4 in the hypothalamus and hypophysis of dogs after the intramuscular injection of T_4

	No. of dogs	Wet tissue weight (mg)*	T ₄ concentration		T ₃ concentration	
			(μg)*	(μg/mg tissue weight)*	(μg)*	(μg/mg tissue weight)*
Posterior hypothalamus						
T ₄ injected	8	795±38	0.042±0.005	0.53 ±0.04×10 ⁻⁴	0.003±0.002	0.04 ±0.01×10 ⁻⁴
Control	5	786±20	0.039±0.007	0.50 ±0.01×10 ⁻⁴	0.004±0.002	0.05 ±0.02×10 ⁻⁴
Medial hypothalamus						
T ₄ injected	7	821±19	0.058±0.002	0.70 ±0.02×10 ⁻⁴	0.005±0.03	0.05 ±0.02×10 ⁻⁴
Control	5	823±12	0.056±0.003	0.68 ±0.03×10 ⁻⁴	0.005±0.03	0.05 ±0.02×10 ⁻⁴
Anterior hypothalamus						
T ₄ injected	8	629±53	0.125±0.026	1.98 ¹ ±0.43×10 ⁻⁴	0.193±0.072	3.05 ² ±0.11×10 ⁻⁴
Control	5	570±24	0.063±0.005	1.13 ¹ ±0.09×10 ⁻⁴	0.061±0.021	1.06 ² ±0.03×10 ⁻⁴
Hypophysis						
T ₄ injected	9	138±10	0.135±0.02	9.75 ³ ±0.14×10 ⁻⁴	0.064±0.008	4.64 ⁴ ±0.55×10 ⁻⁴
Control	5	135± 6	0.044±0.001	3.25 ³ ±0.09×10 ⁻⁴	0.016±0.005	1.18 ⁴ ±0.35×10 ⁻⁴

* Mean $\pm$ Standard error

The differences between the pairs values of individual group in Student's t-test; 1:P<0.05, 2:P<0.02, 3:P<0.03 and 4:P<0.02.

Table 4. Concentration of PBI, T_3 , T_4 and TRF in the peripheral venous blood of dogs after the injection of T_4

	No. of dogs	PBI (μ g%)*	T_4 (μ g%)*	T_3 (μ g%)*	TRF [†] (TSH, J.S.U.%)*
Before injection	5	2.5 $\pm$ 0.3	2.0 $\pm$ 0.2	0.3 $\pm$ 0.1	0.25 $\pm$ 0.01
2 hrs. after injection	5	1.5 $\pm$ 0.2	1.1 $\pm$ 0.1	0.2 $\pm$ 0.1	0.14 $\pm$ 0.005

* Mean $\pm$ Standard error[†] TRF value like as shown in Table 2.

DISCUSSION

Previously the authors succeeded in extracting, from the anterior hypothalamus, TRF, a neurohumor which controls the release of TSH (Shibusawa *et al.*, 1956a, 1959a).

It was later confirmed that when T_4 or T_3 was locally administered into the anterior hypothalamus, the release of TRF was inhibited (unpublished data). T_4 and T_3 were subsequently considered to take part in feed back mechanism together with TRF, controlling in this way the secretion of pituitary TSH. In the present experiments the authors attempted to ascertain further the feed back relation between thyroid hormones and TRF at the level of the anterior hypothalamus.

Many workers have already recognized the accumulation of ^{131}I in the neurosecretory system such as the median eminence, paraventricular nucleus, and neurohypophysis when ^{131}I -L- T_4 and ^{131}I -L- T_3 were administered. For example, Ford and Gross (1958a) reported that when ^{131}I - T_4 and - T_3 were injected into

rabbits, the highest accumulation was seen in the neurohypophysis, and next in the adenohypophysis, while the concentrations in the hypothalamus were equal to those in the pons and medulla. In similar experiments with guinea pigs they also observed accumulation of ^{131}I in the descending order of the adenohypophysis, tuber cinereum, and neurohypophysis.

In the authors' results in dogs, T_4 concentration in the anterior hypothalamus showed an evident increase at 2 hrs. after the injection of T_3 . In the hypophysis, T_4 concentration displayed no change, while T_3 showed a significant increase. The injection of T_3 did not produce any change in T_3 and T_4 concentration in the medial and posterior hypothalamus. On the other hand, the injection of T_4 increased T_4 concentration in the anterior hypothalamus nearly twice and T_3 nearly to 3 times. Since these results were obtained with dogs, difference from those by Ford *et al.* (1957), which were obtained with guinea pigs and rabbits, might be expected. Nevertheless the increase of T_3 and T_4 in the anterior hypothalamus seemed to correspond with the earlier results that TRF was produced in the anterior hypothalamus, and to attain the posterior lobe of the hypophysis through the neurosecretory tract (Shibusawa *et al.*, 1959b, c). It is therefore highly probable that exogenously given T_3 or T_4 might be linked with TRF in the feedback type in the neurosecretory system of the hypothalamus. When T_3 and T_4 concentrations in the anterior hypothalamus were increased their concentrations in peripheral venous blood would fall as the consequence.

Von Euler and Holmgren (1956) observed that L-T_4 , injected into the adenohypophysis of the rabbit, inhibited the liberation of ^{131}I from the thyroid, while the same hormone in the same dose was ineffective when injected into the hypothalamus and the median eminence. On this ground they concluded that T_4 has a feedback relation with TSH solely at the level of the hypophysis. Florsheim (1958), who carried out experiments with rats, considered the thyroid-pituitary feedback system controlling the secretion of TSH to be independent on the hypothalamus. However, Yamada and Greer (1959) reported the inhibition of thyroidal ^{131}I uptake by T_4 injected into the anterior hypothalamus and the anterior lobe of the hypophysis, but the injection into the medial hypothalamus or the anterior lobe of the hypophysis showed no such effect. These reports suggest the participation of the anterior hypothalamus in the pituitary-thyroid feedback system. The authors found that when T_3 or T_4 was injected into the anterior hypothalamus or the anterior lobe of the hypophysis, blood concentration of TRF decreased, probably due to the effect of thyroid hormones at the hypothalamic level. In the present series of experiments of exogenous administration of T_3 and T_4 , the rise in the concentrations of T_3 and T_4 in the systemic circulation resulted in an inhibition of the release of TRF from the anterior hypothalamus consequently leading to the decrease in the release of TSH from the hypophysis and of T_3 and T_4 from the thyroid. TSH secretion thus appears to be not only controlled by the thyroid-pituitary feedback relationship at the level of the hypophysis, but also supplemented by thyroid-TRF feedback mechanism at the level of the anterior hypothalamus.

SUMMARY

(1) After the intramuscular injection of T_3 , T_4 was increased in the anterior hypothalamus, and T_3 in the hypophysis.

(2) Two hrs. after the intramuscular injection of T_4 , 2 fold increase of T_4 and 3 fold of T_3 were seen in the anterior hypothalamus, while in the hypophysis, T_4 increased 3 times, and T_3 4 times. No change was observed in the medial and the posterior hypothalamus.

(3) T_3 and T_4 are considered to constitute feedback type mechanism with TRF and TSH, respectively.

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