EFFECTS OF THE "THYROTROPHIN RELEASING PRINCIPLE (TRF)" AFTER THE SECTION OF THE PITUITARY STALK

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In the preceding number of this journal, we were able to demonstrate that the humoral factor from the anterior hypothalamus has a powerful action in stimulating the liberation of the hypophyseal thyrotrophin and in increasing the weight of the thyroid. The pituitary function has recently been examined under experimental condition following the section of the pituitary stalk (Harris, 1950; Barnett, 1951). Though our "Humoral Factor (TRF)" seemed likely to be released from the hypothalamic neurosecretory system in blood, question arose whether the stimulating activity of the TRF in liberating the pituitary thyrotrophin (TSH) will remain even in the stalk-divided animals.

The object of this paper is to clear the question, casting the credit for hypothalamic control over the pituitary thyroidal axis.

MATERIALS AND METHODS

Adult male mongrel dogs weighing 15-17 kg were used throughout the experiments. They were maintained in a good environment and fed with good diet. Section of the pituitary stalk was performed by the temporal approach according to the description of Harris (1950).

Experimental animals were divided into the following groups:

Group 1: Dogs with stalk section alone (control group).

Group 2: Following the stalk section, dogs were given our Hypothalamic Extract, intramuscularly.

Group 3: Following the stalk section, dogs were given our Hypothalamic Extract and chlorpromazine simultaneously. Chlorpromazine has been proved to reduce the stress response in surgery and was used in our experiments in order to inhibit the hypothalamic neurosecretion (Shibusawa and Saito, 1955, a. b. c).

Group 4: Dogs with stalk section and thyroidectomy.

Examinations were made in the estimation of the thyroidal uptake of I^{131} , thyroidal weight, serum protein-bound iodine (PBI), and blood thyrotrophin (TSH) content. Uptake of radioactive iodine was determined by the method of Adams and Purves (1955), and TSH content of the blood was determined by the β -counting of thyroidal preparation as compared with standard thyrotrophin (Armour Laboratories).¹ The neurosecrets were stained by the Gomori's method, and anterior pituitary cells were stained with Azan and PAS methods.

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RESULTS

Thyroidal weight

The average weight of the thyroid glands of stalk-sectioned dogs was less than that of unoperated controls. With the continued use of our Hypothalamic Extract subcutaneously or intramuscularly, the thyroids of operated dogs markedly increased in weight (Table 1). In the group of combined uses of our Hypothalamic

Table 1. Effects of Stalk Section and TRF on Thyroid Weights of male Dogs.

Group	Dogs	Thyroid weight mg/kg	
		Before	Ten days after stalk section
1	Stalk section alone (5)	112±6.7	93±8.3
2	Stalk section+TRF (5)	105±7.3	122±11.5
3	Stalk section+TRF and chlorpromazine (5)	104±9.4	95±7.7

Extract (TRF) and chlorpromazine, however, the thyroidal weight did not increase, but showed a tendency to decrease. This effect of TRF was not changed by the mode of administration and showed the same results both in the cases of TRF-injection immediately after stalk section and in the cases of TRF-injection on the fifth day following operation. About the thyroidal weight of stalk-sectioned animals, Barnett and Greep (1951) reported the results similar as ours.

Thyroidal uptake of I^{131} .

In table 2, thyroidal I^{131} uptake in various conditions are shown. The I^{131} uptake of stalk-sectioned dogs (Group 1) decreased day by day. In contrast to this, the I^{131} uptake of stalk-sectioned dogs with combined administration of TRF

Table 2. Thyroidal Uptake of I^{131} after Stalk Section

Group	Dogs	Thyroidal uptake of I^{131} %					
		Days					
		Before	2	4	6	8	10
1	Stalk section alone (5)	17.5	24.1	10.1	12.0	10.4	9.4
2	Stalk section+TRF (5)	16.4	26.1	28.0	18.4	18.6	20.5
3	Stalk section+TRF and chlorpromazine (5)	16.7	27.7	21.6	17.0	16.1	13.0

(Group 2) did not decrease but increased for about ten days following the operation. Simultaneous injection of TRF and chlorpromazine (Group 3) did not cause the increased up-take of I^{131} but rather decreased in the same manner as the stalk-sectioned alone (Group 1). Thus, the increase of thyroidal I^{131} uptake is thought to indicate the pituitary-thyroidal hyperfunction.

Blood concentration of TSH

Table 3 shows the blood TSH concentration in the stalk-divided dogs. Blood TSH concentration increased by the TRF injection even after stalk section and continued for the administration period. Combination procedure of stalk-section

Table 3. Blood Concentration of TSH after Stalk Section

Group	Dogs	Blood concentration of TSH $\mu\text{g/ml}$					
		Days					
		Before	5	7	9	11	13
1	Stalk section alone (5)	31	35	91	96	17	20
2	Stalk section+TRF (5)	28	24	75	95	68	35
3	Stalk section+TRF and chlorpromazine (5)	28	42	78	63	10	21
4	Stalk section+thyroidectomy (5)	27	16	92	75	50	30

and TRF injection increased the degree of the elevation of TSH level in the continuation period. On the other hand, combined use of chlorpromazine inhibited the TRF effect and reduced the activity intensely.

In Group 4, in which the stalk was sectioned and the thyroid was removed, blood TSH level increased as remarkably as the TRF injected group.

PBI levels did not change remarkably in any of the four groups. Namely, PBI level is not influenced by the use of TRF or chlorpromazine.

Histological findings

Histological observations were made in four experimental groups. Invariably there are gross and microscopic changes in the pituitary gland as a consequence of stalk section. The gland was shrunken and weighed less than those of the unoperated control. The chromophils became atrophic and anilin blue-stained granules were seen richly between the cells (Fig. 1). In Group 2, basophils increased in number and size, and the grouped cells, which were called σ -cells by Halmi (1951), were seen abundantly (Fig. 2).

In thyroid glands, the average height of the epithelial cells in stalk-sectioned dogs was reduced as compared to that of control (Fig. 3). In Group 2, the epithelium showed hypertrophy and resembled those of TSH-administered group, which were reported by us in the preceding number of this journal. These results show that in the recently stalk-divided dogs an exogenous administration of TRF stimulates the liberation of the pituitary thyrotrophin.

Changes in the hypothalamic neurosecretory system are of noteworthy. The secretory cells of the Nucleus paraventricularis and supraopticus showed a rich holding of the gomorphil substances after stalk section (Figs. 4, 5), and on the contrary, neurohypophysis contained few gomorphil granules, indicating the disconnection of the tractus supraoptico-, paraventriculo-hypophyseus, along which neurosecrets stream to the posterior pituitary (Fig. 6). These changes were similar in all groups but 4, in which the ganglion cells of the Nucleus supraopticus and paraventricularis showed the marked release of the gomorphil substances (Fig. 7).

DISCUSSION

The influence exerted by the anterior hypothalamus on the adenohypophysis has been the subject of several reviews in the last few years (Cheng *et al.* 1949; Greep and Barrnett, 1951; Anand and Dua, 1955). The hypothalamic neurose-

cretory system has been proved to play an important role in the liberation of trophic hormones of the adenohypophysis (Mirsky, 1954; Martini, 1955). We also proposed the stimulatory effects of the hypothalamic neurosecretion on the adenohypophysis in surgical stress.

Namely, since 1953, evidences have been found in our laboratory that vasopressin may serve as the neurohumor involved in the final link for the liberation of the adrenocorticotrophic hormone and oxytocin responsible for the gonadotrophin (Shibusawa *et al.* 1954, 1955, 1956). Although the vascular integrity between the hypothalamus and the anterior lobe of the pituitary gland has been investigated for a long time, there exists a lack of unanimity of opinions regarding the humoral agents regulating the adenohypophysial secretion, being transported through the vascular connection.

The existence, site, and direction of a stream of vascular connections, the portal system, transporting blood from the hypothalamus to the anterior lobe, are clearly demonstrated (*e. g.* Green and Harris, 1947; Harris and Jacobsohn (1952) and Jacobsohn (1956), using the pituitary transplants in the ocular chambers in experimental animals, suggested that the function of the anterior pituitary is dependent upon vascular connections between the hypothalamus and the pituitary. On the other hand, the effect of the destructive lesions in different parts of the hypothalamus was reported by several investigators with regard to the release of ACTH and GTH in response to stressful conditions of experimental animals (De Groot and Harris, 1952; Hume and Wittenstein, 1950; Porter, 1953; McCann, 1953; McCann and Sydnor, 1954; Ganong and Hume, 1954; Keller *et al.*, 1954; Laquer *et al.*, 1955; Bogdanove, 1955; and Martini, 1956).

Experiments now in progress in our laboratory show that a humoral factor extracted from the anterior hypothalamus and urine stimulates the liberation of thyrotrophin (TSH) and growth of the thyroid.

The results obtained here indicate that our TRF, which is produced as a neurosecrete by the anterior hypothalamus and is transported via the portal vessels, remains possible to stimulate the release of TSH and responsible for the activation of the pituitary-thyroidal axis even after the disconnection of the stalk. TRF is thus regarded as a neurosecretory product of the hypothalamus, similarly as vasopressin and oxytocin (*cf.* other paper in preceding number), and passes via the portal vessels to the receptive cells of the adenohypophysis. As a hypothalamic humoral agent, TRF is reckoned to be involved in the natural mechanism(s) regulating the secretion of the thyrotrophin.

Administration of TRF can maintain the thyroidal uptake of I^{131} , blood level of TSH and the weight of thyroid even after the division of the pituitary stalk, and without TRF a marked atrophy of the thyroid takes place within 2 weeks.

SUMMARY

After the disconnection of the pituitary stalk in dogs, weight of the thyroid, uptake of radioactive I^{131} , and blood level of TSH decreased within 2 weeks. The continued administration of our Hypothalamic or Urine Extract, containing a high potency of the thyrotrophin-releasing action, was capable of maintaining

the pituitary-thyroidal functions in the normal range. These findings were also confirmed by histological examination. The activity of this extract was diminished by the combined use of chlorpromazine.

When animals were thyroidectomized following the section of the pituitary stalk, the hypothalamic neurosecretory function increased, and the beta cells of the adenohypophysis increased in number and size and showed the augmentation of thyrotrophic function. The humoral agent of the hypothalamus involved in the final step of the regulation over the pituitary-thyroidal system was discussed briefly.

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Fig. 1. Adenohypophysis; the gland is shrunken and cells become atrophic. After stalk section (Azan staining $\times 840$).

Fig. 2. Adenohypophysis; stalk section and TRF injection. Beta cells increase in number and become hypertrophic (Azan staining $\times 1400$).

Fig. 3. Thyroid gland; flat epithelium and state of hypofunction. After stalk section (H. E. $\times 420$).

Fig. 4. Nucl. supraopt.; gomoriphil granules are found abundantly. After stalk section (Gomori's aldehyde fuchsin staining $\times 125$).

Fig. 5. Nucl. supraopt.; rich in gomorphil granules ($\times 840$). (cf. Fig. 4)

Fig. 6. Neurohypophysis; few gomorphil granules. After stalk section ($\times 185$).

Fig. 7. Nucl. supraopt.; releasing of gomorphil granules. After stalk section and thyroidectomy ($\times 560$).

Fig. 8. Thyroid gland; administration of TRF after the section of the stalk. ($\times 840$).