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THYROXINE FEED-BACK ON THE REGULATION OF THYROTROPHIN (TSH) SECRETION*

BY J. N. PANDA AND C. W. TURNER

From the Department of Dairy Husbandry and the Space Science Research Center, University of Missouri, Columbia, U.S.A.

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SUMMARY

1. The role of thyroid hormone as a feed-back at the hypothalamic level in the control of thyrotrophin (TSH) secretion and release has been investigated by estimating the plasma and pituitary TSH levels following intrahypophysial and intrahypothalamic thyroid autotransplants.

2. Thyroidectomized rats bearing thyroid autotransplants in the pituitary had a significantly lower ($P < 0{\cdot}001$) plasma TSH than that of controls at 26° C but not at 4° C.

3. Thyroidectomized rats bearing thyroid autotransplants in the supraoptic area showed a significantly lower ($P < 0{\cdot}001$) level of plasma TSH and higher pituitary TSH at 4° C but not at 26° C.

4. Study with both unilaterally and bilaterally thyroidectomized rats bearing thyroid autotransplants either in the pituitary or in the hypothalamus revealed that thyroxine feed-back operates at pituitary level in normal situations (26° C) and there exists a feed-back through higher centres, specifically the TSR secreting area of the hypothalamus, in situations demanding higher thyroid function, as in cold exposure.

INTRODUCTION

It is quite well established that the circulating level of thyroid hormone plays an important role in regulating the basal secretion of TSH through a feed-back mechanism of the thyroid-pituitary axis (Hoskins, 1949). This feed-back theory fails to explain the increase of circulating thyroid hormone and presumably of plasma TSH following electrical stimulation of the hypothalamus (Harris & Woods, 1958). Moreover, it has been reported that cold exposure of rats causes significant increases both in thyroid hormone secretion rate (Bauman & Turner, 1967) and plasma and pituitary TSH levels (Panda & Turner, 1967*a*). The fact that the thyroid

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hormone has general and specific effects on neural tissues has led many investigators to search for a site, if any, in the hypothalamus responsive to thyroid hormone with respect to the control of TSH secretion. Jensen & Clark (1951) observed that following intravenous administration of [^{131}I]thyroxine, the median eminence, neural lobe and infundibulum of the rabbit selectively took up [^{131}]I suggesting the involvement of the posterior pituitary in the thyroid hormone feed-back. Ford & Gross (1958) observed that the distribution of [^{131}I]thyroid hormone in the brain and pituitary of rabbits was quite similar with accumulations highest in the median eminence and supra-optic-paraventricular regions. Chueng-Shyang (1963) reported that the [^{131}I]thyroidal uptake in hypophysectomized cockerels with pituitary autotransplants was decreased and was proportional to the amount of thyroxine injected and concluded that the feedback mechanism of thyroid hormone is active even if the hypophysis is removed from the hypothalamus.

Micro-injections of thyroid hormone into various hypothalamic areas have been used to study the involvement of the thyroid hormones in hypothalamic regulation of TSH secretion. Euler & Holmgren (1956) found that the receptor sensitive to thyroxine in the process of thyroxine-induced inhibition of TSH release was located in the adenohypophysis. Yamada & Greer (1959) and Yamada (1959) showed by utilizing [^{131}I]-thyroidal release to evaluate TSH secretion that micro-injections of thyroxine into the anterior pituitary and TSH-regulating area of the anterior hypothalamus of the rat produced significant inhibition of TSH release. Similar injections into the subarachnoid space and into posterior or preoptic hypothalamic areas were without effect. Hypothalamic injections had a longer latent period than those into the pituitaries and this suggested that there are two separate mechanisms concerned with the release of TSH, one directly through the pituitary and the other through the central nervous system. Harrison (1961) contradicted the presence of a hypothalamic receptor sensitive to thyroxine. Bogdanove & Crabill (1961) observed that small pieces of thyroid tissue when transplanted directly into the anterior pituitary prevented the development of thyroidectomy cells in thyroidectomized rats. Similar autotransplantations made into specific areas of the hypothalamus showed a less pronounced inhibition of thyroidectomy reaction. They suggested that thyroxine might diffuse from the transplants in the hypothalamus into the pituitary where actually the receptors are located. The interpretation that the inhibition from thyroid transplants in the hypothalamus resulted from a direct thyroid effect on the pituitary thyrotrophs and acidophiles was offered instead of the alternative idea that the hypothalamus forms part of a slowly reacting thyroid-pituitary feed-back circuit.

Considering such controversial reports, the present study was designed to localize the mode and extent of action of thyroid hormone on the hypothalamus-pituitary-thyroid axis in providing a feed-back control of TSH secretion. Since it has been reported that the supra-optic area of the hypothalamus is concerned directly with TSH secretion and release (Panda & Turner, 1967*b*) from the adenophyophysis, autotransplantations of thyroid fragments were made in this area of the C.N.S. to study the presence of a receptor sensitive to thyroid hormone feed-back in the control of TSH release. The response was studied by direct estimation of pituitary and plasma TSH.

METHODS

Adult female Sprague–Dawley–Rolfsmeyer rats weighing 200–250 g were used in this investigation. The animals were housed in separate cages in a constant environment of either 25 or 4° C with daily exposure to 14 hr of light and 10 hr of darkness. They were fed Purina Lab Chow and water *ad libitum*. The experimental and control animals were sacrificed at 7 a.m. and the blood (about 5 ml.) was collected, in sterile syringes with solid heparin as anticoagulant, from the dorsal aorta under ether anaesthesia and immediately centrifuged. The plasma was stored in sterile vials at −20° C until used. The pituitaries were collected on ice immediately after decapitation, weighed and homogenized with sterile cold saline and centrifuged. The supernatant was stored at −20° C until used. The assay technique employed to estimate plasma and pituitary TSH was exactly the same as reported from this laboratory (Panda & Turner, 1967*b*) with the exception that this time the bovine TSH preparation (Bovine Thyrotrophin (NIH-TSH-B-4) kindly supplied by NIH, Bethesda, Maryland), used both for antibody production and standards, was of the potency of 40 USP units/10 mg. The contamination of this preparation with luteinizing hormone (LH) was of the order of 18 m-u/mg.

Estimation of plasma volume of adult rats was based upon the observations of Wang (1959) of 3·79 ml./100 g body wt. The total circulating TSH was calculated by multiplying the TSH/ml. of plasma with the total plasma volume. This amount of TSH was used to calculate the percentage of the total pituitary TSH.

Thyroidectomy. Thyroidectomy was performed surgically under ether anaesthesia as described by Ducommun (1962). This procedure involved the separation of the recurrent laryngeal nerve from the thyroid capsule and subsequent removal of both the thyroid lobes and isthmus. Thyroidectomized animals were given 1 % calcium gluconate in drinking water. Tests for residual thyroid tissue were made by ^{131}I uptake method and at post-mortem.

Autotransplantation of thyroid tissue into the pituitary and hypothalamus. Thyroid glands were aseptically dissected out and put in ice-cold saline until transplanted into the hypothalamus within 3–5 min of excision. The autotransplants, weighing approximately 2 mg, were delivered into the supra-optic area with the aid of a stereotaxic apparatus and placed bilaterally in symmetrical positions. The delivery of the tissue to the proper site was made as follows: the co-ordinates in the stereotaxic apparatus to reach the supra-optic area were determined following the stereotaxic atlas of the rat brain by König & Klippel (1963). Then a sterile glass capillary 1 cm long was introduced to encircle the electrode and the thyroid fragment was attached to the tip of the electrode just below the lower end of the capillary tube. The transplant was then driven into the brain to reach the desired area which was already fixed in suitable position in the apparatus. After the tissue reached the proper site, the glass capillary was left in place but the electrode was withdrawn. Only two animals

developed a reaction and were discarded. Glass capillaries were inserted for the same length of time in the controls. No other tissue was used to serve as a control.

Thyroid autotransplants into the hypophysis were made by drilling a small hole through the spenoid bone by the parapharyngeal approach. Then the hypophysial capsule was ruptured and small fragments (2–3) of thyroid tissue were transferred immediately into the pituitary from the normal site and the hole was plugged by a small piece of neck muscle, thyroidectomy completed and skin sutured. The animals were allowed to recover for 10–15 days and the survivors used for study.

The brains of these animals were examined histologically and those whose brain picture showed thyroid cells in the supra-optic area were used for the study. Animals showing scars in the site were discarded.

RESULTS

Totally thyroidectomized animals maintained at $26 \pm 1°$ C had a plasma TSH level of $1{\cdot}56 \pm 0{\cdot}08$ m-u./ml. and a total circulating level of 3·25 % of the pituitary TSH content of $49{\cdot}60 \pm 4{\cdot}26$ m-u./mg. When thyroid tissue was autotransplanted in the supra-optic area of the hypothalamus and the rats maintained in similar fashion, the plasma TSH level $1{\cdot}64 \pm 0{\cdot}05$ m-u./ml. and the pituitary content $38{\cdot}78 \pm 3{\cdot}74$ m-u./mg showing no significant difference from the controls.

Partially thyroidectomized animals kept at $26 \pm 1°$ C had a plasma TSH level of $0{\cdot}98 \pm 0{\cdot}11$ m-u./ml. with a total circulating level of 2·09 % of the pituitary TSH content of $52{\cdot}28 \pm 4{\cdot}67$ m-u./mg. The thyroid weight was $10{\cdot}2 \pm 0{\cdot}22$ mg. When thyroid tissue from such animals was transplanted in the rectus abdominis muscle, the plasma TSH level was found to be $0{\cdot}88 \pm 0{\cdot}06$ m-u./ml. with a total circulating level of 1·64 % of the pituitary TSH content of $66{\cdot}17 \pm 4{\cdot}67$ m-u./mg and the thyroid weight was $7{\cdot}5 \pm 0{\cdot}59$ mg. When thyroid tissue was transplanted in the supra-optic area of the brain, the plasma TSH level was $1{\cdot}11 \pm 0{\cdot}05$ m-u./ml. with a total circulating level of 2·16 % of the pituitary TSH of $44{\cdot}65 \pm 2{\cdot}86$ m-u./mg. The thyroid weight was $9{\cdot}3 \pm 0{\cdot}48$ mg.

A group of rats unilaterally thyroidectomized with a hole drilled through the sphenoid bone but with no thyroid tissue transplant was maintained at 26° C. They showed a plasma TSH level of $0{\cdot}89 \pm 0{\cdot}06$ m-u./mg with a pituitary TSH of $57{\cdot}53 \pm 3{\cdot}30$ m-u./mg and a hypertrophied thyroid of $9{\cdot}1 \pm 0{\cdot}08$ mg. A similar group with thyroid tissue autotransplanted into the pituitary showed a significantly lower ($P < 0{\cdot}005$) plasma TSH level of $0{\cdot}62 \pm 0{\cdot}06$ m-u./ml. with a heavier pituitary weight of $11{\cdot}2 \pm 0{\cdot}86$ mg and a markedly lower thyroid weight of $6{\cdot}3 \pm 0{\cdot}41$ mg indicating absence of normal compensatory thyroid hypertrophy. Animals after total thyroidectomy and bearing a thyroid autotransplant into the pituitary had similarly a significantly lower plasma level of TSH of $0{\cdot}74 \pm 0{\cdot}06$ m-u./ml. (Table 1).

Totally thyroidectomized rats maintained at 4° C for 10 days showed a

TABLE 1. Plasma and pituitary TSH levels of rats under different experimental conditions exposed to 20° C for 10 days

Group no.	Treatment	No. of rats	Final body wt. mean (g)	Thyroid weight mean ± S.E. (mg)	Pituitary weight mean ± S.E. (mg)	TSH/ml. of plasma mean ± S.E. (m-u.)	TSH/pituitary gland mean ± S.E. (m-u.)	TSH/mg pituitary mean ± S.E. (m-u.)
1	Control. Bilaterally thyroidectomized	6	230	—	8·5 ± 0·19	1·56 ± 0·08[1]	418·03 ± 11·00	49·60 ± 4·26
2	Bilaterally thyroidectomized; thyroid transplanted in the brain	6	239	—	10·0 ± 0·14	1·64 ± 0·05[2]	387·87 ± 22·00	38·78 ± 3·74
3	Control. Unilaterally thyroidectomized	5	255	10·2 ± 0·22	8·6 ± 0·16	0·98 ± 0·11[3]	454·10 ± 24·09	52·28 ± 4·67
4	Unilaterally thyroidectomized; thyroid transplanted in brain	8	241	9·3 ± 0·48	10·5 ± 0·15	1·11 ± 0·05[4]	468·85 ± 27·88	44·65 ± 2·86
5	Unilaterally thyroidectomized; thyroid in muscle	5	265	7·5 ± 0·59	8·1 ± 0·08	0·88 ± 0·06	538·34 ± 12·04	66·17 ± 4·67
6	Unilaterally thyroidectomized; drilled at pituitary	4	231	9·1 ± 0·08	7·7 ± 0·26	0·89 ± 0·06[5]	443·00 ± 10·20	57·53 ± 3·30
7	Unilaterally thyroidectomized; thyroid transplanted in pituitary	8	216	6·3 ± 0·41	11·2 ± 0·86	0·62 ± 0·06[6]	—	—
8	Bilaterally thyroidectomized; thyroid transplanted in pituitary	7	218	—	10·1 ± 0·69	0·74 ± 0·06[7]	—	—

TSH = Thyrotrophin.

S.E. = Standard error of the mean.

All calculations were made from the mean of duplicate assays.

Student's t test was used for test of significance.

1 *vs.* 2 not significant; 3 *vs.* 4 not significant; 3 *vs.* 5 not significant; 5 *vs.* 6 ($P < 0{\cdot}005$) significant; 1 *vs.* 7 ($P < 0{\cdot}001$) significant.

plasma TSH level of 2·95 ± 0·25 m-u./ml. with a total circulating level of 5·54 % of the pituitary TSH of 51·47 ± 2·19 m-u./mg. Such animals, when bearing a thyroid autotransplant in the supra-optic area of the brain and kept at the same temperature, showed a significantly lower ($P < 0·001$) plasma TSH level of 1·02 ± 0·10 m-u./mg and a significantly higher pituitary TSH content of 146·45 ± 4·66 m-u./mg only 0·54 % of the total being released to the circulation.

Animals unilaterally thyroidectomized and kept at 4° C for 10 days had a plasma TSH level of 2·57 ± 0·17 m-u./ml. with a circulating level of 2·67 % of the total pituitary TSH content of 85·44 ± 17·52 m-u./mg. The thyroid weight was 17·9 ± 0·09 mg. Such animals, bearing a thyroid autotransplant in the supra-optic area, showed a significantly lower ($P < 0·001$) plasma TSH of 0·90 ± 0·05 m-u./ml. with a pituitary TSH of 103·89 ± 4·39 m-u./mg, and a much smaller thyroid of 10·4 ± 0·71 mg. When a thyroid autotransplant was placed in the muscle, the animals showed plasma TSH of 1·83 ± 0·05 m-u./ml., a pituitary level of 95·53 ± 6·44 m-u./mg and a thyroid weight of 9·9 ± 0·91 mg.

A group of rats unilaterally thyroidectomized with a hole drilled through the sphenoid bone, but without a thyroid tissue transplant and maintained at 4° C for 10 days, had a plasma TSH of 2·41 ± 0·22 m-u./mg and pituitary TSH of 84·73 ± 3·67 m-u./mg. Such animals bearing a thyroid autotransplant in the pituitary showed a plasma TSH of 2·28 ± 0·18 m-u./ml. with a thyroid weight of 15·4 ± 0·53 mg. Totally thyroidectomized animals, bearing a thyroid autotransplant in the pituitary, had a plasma TSH level of 2·79 ± 0·18 m-u./ml. (Table 2).

DISCUSSION

In the present study it was found that bilaterally thyroidectomized rats, bearing thyroid autotransplants in the pituitary and maintained at 26° C, had a significantly lower ($P < 0·001$) plasma TSH of 0·74 ± 0·06 m-u./ml. compared with control thyroidectomized rats having 1·56 ± 0·08 m-u./ml. and thyroidectomized rats, bearing intrahypothalamic thyroid autotransplants, with a plasma TSH level of 1·64 ± 0·05 m-u./ml. Moreover, intrapituitary transplants of thyroid tissue prevented the compensatory hypertrophy of the remaining thyroid in the unilaterally thyroidectomized rats due to a lower level of plasma TSH of 0·62 ± 0·06 m-u./ml. The controls without a transplant showed a higher plasma TSH level of 0·89 ± 0·06 m-u./ml. with a heavier thyroid of 9·1 ± 0·08 mg. On the other hand, similarly treated animals bearing a thyroid autotransplant in the supra-optic area of the hypothalamus also showed a much higher plasma TSH level of 1·11 ± 0·05 m-u./ml. with a heavier thyroid of 9·3 ± 0·48 mg. Ab-

TABLE 2. Plasma and pituitary TSH levels of rats under different experimental conditions exposed to 4° C for 10 days

Group no.	Treatment	No. of rats	Final body wt. mean (g)	Thyroid weight mean ± S.E. (mg)	Pituitary weight mean ± S.E. (mg)	TSH/ml. of plasma mean ± S.E. (m-u.)	TSH/pituitary gland mean ± S.E. (m-u.)	TSH/mg pituitary mean ± S.E. (m-u.)
1	Control. Bilaterally thyroidectomized	6	232	—	9·10 ± ·22	2·95 ± 0·25[1]	468·44 ± 22·00	51·47 ± 2·19
2	Bilaterally thyroidectomized; thyroid transplanted in brain	7	207	—	10·4 ± 0·29	1·02 ± 0·10[2]	1510·54 ± 29·80	146·45 ± 4·66
3	Control. Unilaterally thyroidectomized	4	204	17·9 ± 0·09	8·7 ± 0·26	2·57 ± 0·17[3]	743·32 ± 20·00	85·44 ± 17·52
4	Unilaterally thyroidectomized; thyroid transplanted in brain	6	221	10·4 ± 0·71	12·1 ± 0·78	0·90 ± 0·05[4]	1257·96 ± 13·42	103·89 ± 4·39
5	Unilaterally thyroidectomized; thyroid in muscle and drilled at pituitary	5	176	9·9 ± 0·91	7·4 ± 0·19	1·83 ± 0·05[5]	708·00 ± 48·24	95·53 ± 6·44
6	Unilaterally thyroidectomized; drilled at pituitary	5	222	13·3 ± 1·28	7·4 ± 0·25	2·41 ± 0·22[6]	627·00 ± 00·00	84·73 ± 3·67
7	Unilaterally thyroidectomized; thyroid transplanted in pituitary	10	222	15·9 ± 0·53	15·3 ± 0·50	2·28 ± 0·18[7]	—	—
8	Bilaterally thyroidectomized; thyroid transplanted in pituitary	7	209	—	12·9 ± 0·89	2·79 ± 0·18[8]	—	—

TSH = Thyrotrophic hormone.

S.E. = Standard error of mean.

All calculations were made from the mean of duplicate assays.

Student's *t* test used for test of significance.

1 *vs.* 2 ($P < 0·001$) significant; 3 *vs.* 4 ($P < 0·001$) significant; 3 *vs.* 6 not significant; 4 *vs.* 5 ($P < 0·001$) significant; 6 *vs.* 7 not significant; 1 *vs.* 8 not significant.

sence of any significant difference between the controls and those with autotransplants in the hypothalamus suggests that the thyroid hormone does not act on the hypothalamus to produce an inhibition of TSH release during normal conditions when a basal level of thyroid function is in demand. On the other hand, it does produce inhibition of TSH release, acting directly on the pituitary under normal situations.

When bilaterally thyroidectomized animals with intrapituitary thyroid autotransplants were maintained at 4° C for 10 days, they showed a plasma TSH level of $2{\cdot}79 \pm 0{\cdot}18$ m-u./ml. comparable with that of the controls ($2{\cdot}95 \pm 0{\cdot}25$ m-u./ml.) and significantly higher ($P < 0{\cdot}001$) than that of rats bearing intra-hypothalamic autotransplants in the supra-optic area with only $1{\cdot}02 \pm 0{\cdot}10$ m-u./ml. A very high pituitary TSH level of $146{\cdot}54 \pm 4{\cdot}66$ m-u./mg was observed in these animals, compared with $51{\cdot}47 \pm 2{\cdot}19$ m-u./mg of pituitary of the controls. This 3-fold increase indicated that there is both an increase in secretion and a significantly decreased release of TSH from the pituitary. The thyroid hormones inhibited the release of TSH-releasing factor (TSH-RF) without affecting the stimulatory effect of the hypothalamus on TSH secretion by the pituitary. In previous studies (Panda & Turner, 1967*a*, *b*) thyroidectomized rats when exposed to 4° C showed a higher level of plasma TSH associated with a lower level of pituitary TSH.

In the group of unilaterally thyroidectomized rats maintained at 4° C (Table 2), those with a thyroid transplant in muscle had a lower plasma TSH than the controls and the thyroid gland weighed less. This suggests that the thyroid gland is able to secrete its hormones when placed away from its normal site and the level of circulating TSH is sensitive to the level of circulating thyroid hormone irrespective of the anatomical position of the gland. However, the level of plasma TSH in rats with a thyroid transplant in brain was significantly lower ($P < 0{\cdot}001$) than that of rats with a transplant in muscle (cf. groups 4 and 5 in Table 2).

The inhibitory effect of thyroid hormone on the release of TSH through the hypothalamus in cold-exposed animals is more clearly demonstrated from our study on unilaterally thyroidectomized animals. The compensatory hypertrophy of the remaining thyroid in the controls was significantly greater than in rats bearing thyroid autotransplants in the hypothalamus. A similar picture is presented in the plasma TSH levels. Unilaterally thyroidectomized animals in the control group showed a plasma level of $2{\cdot}57 \pm 0{\cdot}17$ m-u./ml. which is comparable with that of rats bearing intra-pituitary thyroid transplants ($2{\cdot}28 \pm 0{\cdot}18$ m-u./ml.), and significantly higher than that of rats bearing intra-hypothalamic thyroid transplants ($0{\cdot}90 \pm 0{\cdot}05$ m-u./ml.). These data suggested that environmental factors do alter the normal thyroid-pituitary feed-back and in

specific conditions, such as cold exposure, a very high level of circulating thyroid hormone exerts its inhibitory effect on the release of TSH by a feed-back mechanism which involves the hypothalamic TSH-RF secreting area.

Perhaps due to this mechanism, after a prolonged exposure to cold, there occurs no further increase in thyroid function, rather there may be an absent or decline to lower levels as reported in rats and other species (Starr & Roskelly, 1940; Leblond, Gross, Peacock & Evans, 1943; Cottle 1960; Johnson, Kibler & Silsby, 1964).

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