Influence of Hypothalamus on Pituitary-Thyroid Axis in the Rat.*† (24528)

WARNER H. FLØRSHEIM

Long Beach Vet. Hospital and UCLA Medical School, Los Angeles, Calif.

We have presented data indicating that lesions in the anterior hypothalamus of the rat produced effects which could be ascribed to reduction in rate of synthesis by the pituitary gland of a single thyrotropic hormone (TSH)(1). Our data did not support Greer's hypothesis(2) which called upon 2 separate thyrotropins, a "metabolic" and a "growth" factor, to explain normal iodide trapping together with reduced hypertrophy after goitrogen treatment in appropriately lesioned rats. Our data showed a reduction in a number of thyroid function criteria. However, we were able to bring only indirect evidence that TSH secretion was decreased by anterior hypothalamic lesions. More direct evidence has been reported by D'Angelo and Traum(3) but the nature of their bioassay makes it impossible to argue convincingly against the dual hormone hypothesis on the basis of their experiments. The current work presents direct evidence that in the lesioned rat there is reduced secretion of "metabolic" TSH. We have further attempted to elucidate the nature of hypothalamic intervention in TSH secretion via the pituitary-thyroid "feedback" mechanism, and we have expanded on previous observations on the effect of hypothalamic lesions on distribution of iodinated amino acids within the thyroid gland.

Methods. Rats used were adult males of Holtzman strain weighing about 250 g at time of lesioning. Large bilaterally symmetrical lesions were produced in the anterior hypothalamic area controlling thyroid function by the procedure previously described(1). Hyperphagia occurred in more than half of the survivors. Rats were used at least a month after lesioning. Propylthiouracil was added to the diet at a level of 0.15% when indicated. Uptake of radioiodine into the thyroid and discharge of radioactivity from this gland were measured as described previously. The thyrotropin biossay employed was developed in this laboratory(4) and is a modification of Gilliland and Strudwick's method measuring discharge of labelled hormone in the chick. Thyroid hormone secretion was measured by estimating graphically the minimum dose of thyroxine given intramuscularly which would completely inhibit discharge of radioactivity from the rat thyroid. Paper chromatography was carried out by the ascending technic at 23° using collidine-water in an atmosphere of ammonia, tert-amylalcohol-acetone-water (20:5:3) and N-butanol saturated with 2 N formic acid. Chromatograms were cut into 1 cm segments at right angles to the direction of migration, and radioactivity determined by means of a well-type scintillation counter.

Results. Table I summarizes results of assaying the pooled sera of 5 "goiter block" and 8 control rats which had been given PTU for 16 days. The serum was administered to chicks in two-1 cc doses. Sera of a group of 5 rats which had been lesioned, but in which the lesions proved to be "ineffective" as to thyroid function by criteria established previously(1), proved to give effects intermediate between "goiter block" and control animals. Since the bioassay employed is based upon a clearly "metabolic" effect, the discharge of labelled hormonal iodine from the thyroid gland, these results show that the level of circulating "metabolic" TSH is lower

TABLE I. Serum TSH Levels in Rats on PTU Diet.

Groups receiving:	No. chicks	% of thyroidal I^{131} discharged
Saline	9	33.9 ± 3.2*
5 mU TSH (USP standard)	10	57.3 ± 4.0
20 *Idem*	10	81.9 ± 3.0
"Goiter block" serum	11	33.3 ± 1.8
"Ineffective" lesion serum	9	45.3 ± 1.1
Unlesioned control "	11	52.7 ± 4.0

* Stand. error of mean.

* We thank Dr. David Solomon for suggesting the experiment testing the relation of hypothalamus to thyroid pituitary feedback system. The technical assistance of Miss Shirley M. Velcoff and Mr. Frank Kelley is gratefully acknowledged.

† Supported in part by grant from U.S.P.H.S.

in goiter block animals than in controls or rats lesioned in areas other than the thyroid controlling one. This difference was significant at the .001 level of confidence.

Unfortunately we have been unable to duplicate these experiments in rats which had not been treated with goitrogens because the levels of TSH in normal serum still appear too low to be determined by available bioassay procedures. Taken in conjunction with our previous findings, the data would seem to obviate the need for assuming the existence of 2 thyrotropins. A single hormone produced under partial hypothalamic control would suffice to explain all data presently available except the work on hypophysectomized mice bearing intraocular pituitary grafts reported by Scow and Greer(5). It may well be that sensitivity to TSH of different aspects of thyroid function varies among different species, and for this reason we are currently reexamining the influence of autografted pituitaries on rat thyroid function in collaboration with Dr. K. M. Knigge.

Von Euler(6) first showed that in the rabbit the site of feedback control of TSH release was in the pituitary rather than the hypothalamus. Brown-Grant(7) postulated that since the majority of hypophyseal blood supply was routed through the hypothalamus, the latter might act as a filter, keeping thyroxine from reaching the pituitary and thus maintaining TSH secretion at a high level. It was therefore of some interest to determine whether appropriate hypothalamic lesions would alter the amount of thyroxine needed to inhibit thyroidal I^{131} discharge completely. In a preliminary experiment 4 out of 15 control rats responded to 2 γ thyroxine a day and 11 to 4 γ. Among lesioned rats 4 out of 14 responded to 2 γ and 10 to 4 γ. In a second experiment using the same animals a month later the stepwise increments were made smaller and a more quantitative estimate was obtained for the minimum effective dose. The effective dose was 2.8 ± 0.2 (standard error) γ for controls and 2.6 ± 0.3 γ for lesioned animals. There was no difference between the 2 groups and we are in agreement with D'Angelo and Traum(8) who also believe that thyroxine inhibits TSH secretion at the pituitary without hypothalamic mediation. The hypothalamus thus would appear to act primarily upon synthesis of TSH rather than its release as claimed by Shibusawa(9), since we previously showed below-normal TSH stores in pituitaries of lesioned animals.

In our previous studies of iodine content of thyroids of normal and lesioned rats, we were unable to show significant differences in I^{127} content between the 2 groups. This was interpreted as arguing against the action of the thyroid-controlling mechanism independent of the pituitary reported by VanderLaan and Caplan(10). However, Blanquet and Tobias(11) have found lesions produced in rat hypothalami by means of a cyclotron beam to cause a striking decrease in thyroid hormone production without concomitant decrease in other organic iodine binding. We have therefore examined pancreatin digests of I^{131}-labelled thyroids from lesioned and control rats by ascending paper chromatography using 3 different solvent systems: collidine-ammonia, tert-amyl alcohol-acetone-water, and n-butanol-formic acid-water. The first 2 systems were used on groups of 9 lesioned and 9 control rats which were killed 24 hours after tracer administration. The third system was used on 19 glands from animals taken 72 hours after tracer administration. In no case could major differences in the distribution of I^{131} between iodide, iodotyrosines and hormonal iodine be observed. This work is being repeated in collaboration with Drs. P. Blanquet and D. Yudilevich using both paper chromatography and their ion exchange method in parallel on the same thyroid digests.

Summary. In rats with anterior hypothalamic lesions the maximal circulating thyrotropin levels after goitrogen feeding are lower than in control animals as measured by bioassay based upon hormonal iodine discharge from chick thyroids. The discharge of labelled hormone from the thyroid of lesioned and control rats is inhibited by equal doses of exogenous thyroxine. This indicates that the thyroid-pituitary feedback system controlling TSH release is independent of the hypothalamus. Finally, in electrolytically lesioned rats with destruction of the thyroid-controll-

ing hypothalamic area there do not appear to be major changes in the relative amounts of iodinated tyrosines and thyronines in the thyroid as have been shown to occur in cyclotron-lesioned rats by Blanquet and Tobias.

1. Florsheim, W. H., *Endocrinol.*, 1958, v62, 783.
2. Greer, M. A., *J. Clin. Endocrinol. and Metab.*, 1952, v12, 1259.
3. D'Angelo, S. A., Traum, R. E., *Endocrinol.*, 1956, v59, 593.
4. Florsheim, W. H., Imagawa, D. T., Greer, M. A., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 664.
5. Scow, R. O., Greer, M. A., *Endocrinol.*, 1955, v56, 590.
6. Von Euler, C., Holmgren, B., *J. Physiol.*, 1956, v131, 125.
7. Brown-Grant, K., *Ciba Colloquia on Endocrinology*, 1957, v10, 97.
8. D'Angelo, S. A., Traum, R. E., *Trans. N. Y. Acad. Sci.*, 1958, v20(II), 244.
9. Shibusawa, K., Saito, S., Nishi, K., Yamamoto, T., Tomizawa, K., Abe, C., *Endocrinol. Japon.*, 1956, v3, 116.
10. VanderLaan, W. P., Caplan, R., *Endocrinol.*, 1954, v54, 437.
11. Blanquet, P., Tobias, C. A., *Clin. Res. Proc.*, 1958, v6, 52.

Received September 30, 1958. P.S.E.B.M., 1959, v100.

Aromatic Amines II. Demonstration of 2-amino-1-naphthyl Glucuronides as Metabolite of 2-naphthylamine in Dog Urine.* (24529)

W. TROLL, S. BELMAN, N. NELSON, M. LEVITZ AND G. H. TWOMBLY

Inst. of Industrial Medicine and Dept. of Obstetrics and Gynecology, N. Y. University-Bellevue Medical Center

Occupational exposure to 2-naphthylamine is recognized as producing bladder cancer in man(1). Attempts to produce bladder cancer in experimental animals with this compound have shown that the dog develops bladder cancer(2,3) with pathology similar to humans(2), while other species such as rabbits, rats, and mice do not(3). Rabbits and rats develop benign tumors but mice show no bladder damage(3). When 2-naphthylamine and some of its metabolites were implanted in the bladder of the resistant mouse, it was found that certain metabolites led to bladder cancer. Of the 7 metabolites tested, 2-amino-1-naphthol hydrochloride(3,4), and 2-amino-1-naphthyl glucuronide(5) were active in producing bladder carcinoma. This observation supports the hypothesis(2) that species specificity is linked to metabolites produced rather than to species difference in sensitivity of bladder tissue. This hypothesis requires that after exposure of 2-naphthylamine the carcinogenic metabolites must be present in urine of susceptible species in significantly higher concentration. The main metabolite of 2-naphthylamine in the dog is 2-amino-1-naphthyl sulfate(6). Since this appears to be inactive in the mouse bladder(4), a search was undertaken for other metabolites in dog's urine. Recent work in this laboratory demonstrated the presence of a previously unreported metabolite in dog urine, bis(2-amino-1-naphthyl) phosphate(7) however, its carcinogenicity has not yet been determined. In the present communication evidence is presented that the dog excretes 3.5-13% of ingested 2-naphthylamine as 2-amino-1-naphthyl glucuronide.

Materials. 1. *2-amino-1-naphthol hydrochloride.* Chromogene Black ETOO (General Aniline and Film Co.) was reduced with sodium hydrosulfite as described by Grandmougin(8). The product was extracted with ether containing a small amount of hydroquinone. The dried ethereal solution was treated with gaseous hydrogen chloride to give the desired product. The infrared spectrum (KBr wafer) was identical with that of a 2-amino-1-naphthol hydrochloride sample generously supplied by the National Aniline Di-

* Aided by grants from Allied Chemical Corp., N.I.H., and Alta R. Prentice Fund.