REGIONAL DISTRIBUTION OF THYROID STIMULATING HORMONE ACTIVITY IN THE PITUITARY GLAND OF THE ATLANTIC STINGRAY, *DASYATIS SABINA*

RODNEY G. JACKSON AND MARTIN SAGE[1]

ABSTRACT

The possibility that the elasmobranch pituitary contains thyroid stimulating hormone (TSH) activity was investigated by measuring the increase in the release of thyroxine from thyroid glands of the Atlantic stingray, *Dasyatis sabina*, incubated with homogenates of various pituitary regions. The ventral lobe of the pars distalis contained most of the TSH activity, with lesser amounts in the neurointermediate lobe. Histological techniques were not sensitive enough to detect changes in the thyroid associated with the increase in thyroxine release. It is concluded that the elasmobranch pituitary contains TSH activity but its functional significance remains to be determined.

Few studies have been conducted to examine the functional relationship between the pituitary and the thyroid gland of elasmobranchs. Dodd and Goddard (unpublished but cited by Dent and Dodd, 1961) hypophysectomized adult dogfish, *Scyliorhinus caniculus*, but found no histological changes in the thyroid after 2 years, whereas Vivien (1964) found that after decapitation of *Scyliorhinus* embryos the thyroid failed to complete its differentiation. The latter result is, of course, open to several interpretations since decapitation removes more than the pituitary. Injection of homoplastic pituitary homogenates into *Scyliorhinus* resulted in histological signs of stimulation of the thyroid gland (Vivien, 1941; Olivereau, 1954). Unfortunately, histological methods of assessing thyroid activity are frequently both insensitive (Sage and Robins, 1970) and unreliable (Swift, 1960).

Ferguson, Dodd, Hunter, and Dodd (unpublished data summarized by Dodd et al. (1963)) using the McKenzie mouse assay found thyroid stimulating hormone (TSH) activity in all parts of the *S. caniculus* pituitary, most of it being in the ventral lobe. However, the highest activity found was much less than that found in the posterior lobe of the mouse pituitary, which presumably does not contain TSH. Their results could be interpreted as suggesting that the small amount of TSH activity found in the dogfish pituitary was of no significance. The interpretation of assays of lower vertebrate TSH on mammalian assay systems is further complicated by the probability of phylogenetic specificity of hormone action. It is known that teleost TSH is relatively inactive on the mammalian thyroid (Fontaine, 1969); similarly it is possible that if there is an elasmobranch TSH it may have low activity on mammalian tissues. In a recent review Gorbman (1969) states that "definite proof of a TSH-like principle in elasmobranch pituitaries remains to be provided." In an attempt to elucidate this problem we investigated the stimulatory effects of homogenates of the different regions of the pituitary of *Dasyatis sabina* on thyroxine release from the animal's own thyroid gland in vitro. This technique eliminates the problem of phylogenetic specificity, and, by measuring thyroxine release, avoids the problems of interpretation associated with histological assessment of thyroid activity.

[1] The University of Texas, Marine Science Institute, Port Aransas, TX 78373.

Manuscript accepted May 1972.
FISHERY BULLETIN: VOL. 71, NO. 1, 1973.

MATERIALS AND METHODS

ANIMALS

Dasyatis sabina (Lesueur) were collected in otter trawls. In the fall and winter stingrays are most abundant in the shallow waters in the Gulf of Mexico adjacent to Port Aransas, Tex. In late spring the stingrays migrate into the bays behind the line of barrier islands where they were caught during the summer (Sage et al., 1972).

INCUBATION TECHNIQUE

Animals were killed by cutting across the hind brain. The compact thyroid is located ventral to the anterior end of the ventral aorta. The thyroid was removed and divided into experimental and control halves, and further divided where necessary so that no piece of tissue was larger than 5 mg. Preliminary experiments indicated that the elasmobranch thyroid was slow in responding to stimulation. Thyroid tissue was therefore incubated for 3 days in 2 ml of elasmobranch saline (Nicoll and Bern, 1964). Antibiotics were added (Bakke et al., 1957) in order to inhibit bacterial growth which might result in the breakdown of the thyroxine released into the medium. The addition of antibiotics does not interfere with the ability of thyroid glands to respond to TSH (Bakke et al., 1957; Sage, 1968a). The incubation flasks were gassed with 95% oxygen and 5% carbon dioxide and shaken at 120 strokes/min at 30°C. This temperature is within the normal environmental range of *D. sabina*. Modification of the incubation medium by the addition of 0.5 mg/ml lactalbumin hydrolysate was found to increase control rates but reduce the variability of the response and was used in later experiments as described in the text.

Homogenates of whole pituitaries or various regions of the stingray pituitary were made in a glass homogenizer and added to the incubation media at a concentration of one pituitary gland or region per thyroid gland. The homogenates were added immediately prior to gassing and adding of the thyroid tissue.

THYROXINE ANALYSIS

At the end of the 3-day incubation period thyroid tissue was removed for histological examination, and the incubation media was centrifuged at 10,000 rcf for 10 min to remove cell debris. Incubations were then stored below 0°C until analyzed. Thyroxine was isolated by ion exchange chromatography (Galton and Pitt-Rivers, 1959). The catalytic effect of iodine in reducing ceric ions was used to quantify thyroxine iodine (Pileggi et al., 1961; Pileggi and Kessler, 1968). Oxford Laboratories' (San Mateo, Calif.) kit[2] of reagents was used in the determinations. The results were converted to rates of thyroxine release per unit thyroid weight per incubation, and the responses of treated halves of the gland were then expressed as a percentage of the matched control incubated halves. Additives to the incubation media were routinely analyzed but were invariably devoid of thyroxine.

HISTOLOGICAL METHODS

At the end of the incubation period, thyroid tissue was removed and fixed in mercuric formol (90 parts saturated mercuric chloride to 10 parts formaldehyde solution). Sections were cut in polyester wax and stained with hematoxylin and light green. The image of the thyroid follicles was projected onto a sheet of paper, and a planimeter was used to determine the percentage of the area of follicle occupied by epithelium.

Unstained thyroid sections were used for interferometric determinations of mass per unit area of the colloid (Bromage and Sage, 1968; Sage, 1968b). Such methods are very sensitive in detecting changes in thyroid activity in both teleosts (Bromage and Sage, 1968) and mammals (Sage and Robins, 1970).

[2] Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

RESULTS

A preliminary study was carried out to determine responsiveness of the thyroid to homogenates of the various regions of pituitary and to control material. The results (Table 1) indicated that the addition of large amounts of protein or protein hydrolysate resulted in a stimulation of the gland, thus suggesting an inadequate culture medium. The medium was therefore modified by the addition of 0.5 mg lactalbumin hydrolysate/ml, and the response to various regions of the pituitary reexamined (Table 2). TSH activity was greatest in the ventral lobe of the proximal pars distalis, but significant activity was also found in the neurointermediate lobe. The latter is not due to the presence of thyroxine in this pituitary lobe since the thyroxine content of the homogenates was undetectable. In this respect the elasmobranch is unlike the mammal where the neural lobe does concentrate thyroxine (see review by Pitt-Rivers and Tata, 1959).

Histological methods have previously been used to assay the state of thyroid activity. In order to determine whether such techniques would detect stimulation resulting from incubation of thyroids with whole pituitary homogenates, interferometric measurements on the colloid were made together with a determination of the percentage of the follicular area occupied by epithelium. Neither technique was sensitive enough to detect the stimulation observed by measuring changes in the release of thyroxine (Table 3).

TABLE 1.—Percentage increase in release of thyroxine from *Dasyatis* thyroid tissue produced by adding homogenates of various regions of the *Dasystis* pituitary or lactalbumin hydrolysate to a medium containing salts, urea, and glucose.

Item	*N*	Mean ± SE
Rostral pars distalis (1 lobe/thyroid)	7	43 ± 22
Neurointermediate lobe (1 lobe/thyroid)	8	*31 ± 9
Proximal pars distalis:		
Dorsal lobe (1 lobe/thyroid)	6	40 ± 18
Ventral lobe (1 lobe/thyroid)	7	47 ± 27
Lactalbumin hydrolysate (2 mg/thyroid)	5	35 ± 24

* Significantly differs from zero, $P<0.01$.

TABLE 2.—Percentage increase in the release of thyroxine from *Dasyatis* thyroid tissue produced by adding homogenates of various regions of the *Dasyatis* pituitary to a medium containing salts, urea, glucose plus lactalbumin hydrolysate.

Item	*N*	Mean ± SE
Rostral pars distalis	9	30 ± 19
Neurointermediate lobe	9	*55 ± 20
Proximal pars distalis:		
Dorsal lobe	9	19 ± 15
Ventral lobe	9	**123 ± 40

* Significantly differs from zero, $P<0.05$.
** Significantly differs from zero, $P<0.02$.

TABLE 3.—A comparison of the effectiveness of various techniques for determining the response of *Dasyatis* thyroid glands to 3-day stimulation in vitro by homogenates of whole *Dasyatis* pituitaries (1 pituitary/thyroid).

Item	*N*	Mean percentage of control values ± SE
Increase in release of thyroxine	12	*51 ± 16
Increase in area of follicles occupied by epithelium	7	4.4 ± 5.1
Decrease in interferometric measure of dry wt/unit area of colloid	12	63 ± 38

* Significantly differs from zero, $P<0.01$.

DISCUSSION

The present work confirms the unpublished but frequently quoted work of Ferguson et al. (Dodd et al., 1963) in that there is TSH activity in the elasmobranch pituitary and that the greatest concentration is found in the ventral lobe where gonadotropic activity has also been found (Dodd, Evennett, and Goddard, 1960). The finding of lesser amounts of TSH activity in the neurointermediate lobe is in agreement with the finding of Goddard and Dodd (unpublished but quoted by Dodd et al., 1960). However, their suggestion that the activity is due to a thyrotropin releasing factor cannot explain the present results obtained in vitro with thyroid tissue. The nature of the neurointermediate thyroid stimulating substance is unknown. Dodd et al. (1963) reported that it is heat stable, whereas the activity of the dogfish ventral lobe is not. However, it is not possible to argue that the activity in the neurointermediate lobe is nonprotein since all the activity present in the frog (*Rana temporaria*) pituitary is heat stable and some at least of this is presumed to be the protein TSH.

The comparison of techniques for the demonstration of TSH activity of the pituitary homogenates on the thyroid clearly indicates the inadequacy of histological methods. In spite of a highly significant increase in the release of thyroxine there was no change observed in the follicular epithelium nor in measurements on the colloid weight per unit area. This method is capable of detecting the response of teleost thyroid follicles to a 24-hr incubation with mammalian TSH (Bromage and Sage, 1968).

While the results of incubations of thyroid with pituitary homogenates indicate TSH activity is present in the pituitary, they do not indicate whether it is of functional significance. An obvious followup to these experiments would be the removal of the ventral lobe and the measurement of blood thyroxine levels. However, removal of the ventral lobe in this species has not so far been possible due to the close association of this region with the carotid anastomosis.

Furthermore, the analysis of thyroxine in elasmobranch blood by the present methods is difficult due to unknown factors in the blood which interfere with thyroxine analysis. From this study we conclude that TSH activity is present in the elasmobranch pituitary and that most of this activity is in the ventral lobe. However the functional significance of this activity remains to be determined.

ACKNOWLEDGMENTS

We are indebted to Mr. J. Thompson and his staff at the Marine Science Institute, especially Boat Captains E. Wingfield and J. Shanklin for help in collecting Atlantic stingrays. We also thank Dr. V. de Vlaming and L. C. Sage for their comments on the manuscript. Supported by NSF grant GB 22995.

LITERATURE CITED

BAKKE, J. L., M. L. HEIDEMAN, N. L. LAWRENCE, AND C. WIBERG.
1957. Bioassay of thyrotropic hormone by weight response of bovine thyroid slices. Endocrinology 61:352-367.

BROMAGE, N. R., AND M. SAGE.
1968. The activity of the thyroid gland of *Poecilia* during the gestation cycle. J. Endocrinol. 41:303-311.

DENT, J. N., AND J. M. DODD.
1961. Some effects of mammalian thyroid stimulating hormone, elasmobranch pituitary gland extracts and temperature on thyroidal activity in newly hatched dogfish (*Scyliorhinus caniculus*). J. Endocrinol. 22:395-402.

DODD, J. M., P. J. EVENNETT, AND C. K. GODDARD.
1960. Reproductive endocrinology in cyclostomes and elasmobranchs. Symp. Zool. Soc. Lond. 1:77-103.

DODD, J. M., K. M. FERGUSON, M. H. I. DODD, AND R. B. HUNTER.
1963. The comparative biology of thyrotropin secretion. *In* S. C. Werner (editor), Thyrotropin, p. 3-38. Charles Thomas, Springfield.

FONTAINE, Y. A.
1969. Studies on the heterothyrotropic activity of preparations of mammalian gonadotropins of teleost fish. Gen. Comp. Endocrinol. Suppl. 2:417-424.

GALTON, V. A., AND R. PITT-RIVERS.
1959. A quantitative method for the separation of thyroid hormones and related compounds from serum and tissues with an anion-exchange resin. Biochem. J. 72:310-313.

GORBMAN, A.
1969. Thyroid function and its control in fishes. *In* W. S. Hoar and D. J. Randall (editors), Fish physiology. Vol. 2, p. 241-274. Academic Press, N.Y.

NICOLL, C. S., AND H. A. BERN.
1964. Prolactin and the pituitary glands of fishes. Gen. Comp. Endocrinol. 4:457-471.

OLIVEREAU, M.
1954. Hypophyse et glande thyroide chez les poissons. Étude histophysiologique de quelques corrélations endocriniennes en particulier chez *Salmo salar* L. Ann. Inst. Oceanogr. Monaco 29: 95-296.

PILEGGI, V. J., AND G. KESSLER.
1968. Determination of organic iodine compounds in serum. IV. A new nonincineration technic for serum thyroxine. Clin. Chem. 14:339-347.

PILEGGI, V. J., D. N. LEE, O. J. GOLUB, AND R. J. HENRY.
1961. Determination of iodine compounds in serum. I. Serum thyroxine in the presence of some iodine contaminants. J. Clin. Endocrinol. Metab. 21: 1272-1279.

PITT-RIVERS, R., AND J. R. TATA.
1959. The thyroid hormones. Pergamon Press, Lond., 247 p.

SAGE, M.
1968a. Assay of mammalian and fish TSH. J. Endocrinol. 41:xv.
1968b. Responses to osmotic stimuli of *Xiphophorus* prolactin cells in organ culture. Gen. Comp. Endocrinol. 10:70-74.

SAGE, M., R. G. JACKSON, W. L. KLESCH, AND V. L. DE VLAMING.
1972. Growth and seasonal distribution of the elasmobranch *Dasyatis sabina*. Contrib. Mar. Sci. 16:71-74.

SAGE, M., AND P. C. ROBINS.
1970. The quantitative relationship between the concentration of TSH and interferometric measurements on the thyroid colloid. Gen. Comp. Endocrinol. 14:601-603.

SWIFT, D. R.
1960. Cyclical activity of the thyroid gland of fish in relation to environmental changes. Symp. Zool. Soc. Lond. 2:17-27.

VIVIEN, J. H.
1941. Contribution a l'étude de la physiologie hypophysaire dans ses relations avec l'appareil génitale, la thyroide et les corps surrénales chez les poissons sélaciens et téléostéens. Bull. Biol. Fr. Belg. 75:257-309.
1964. Influence de la décapitation sur le developpement de l'ébauche thyroidienne de l'embryon de *Scylliorhinus caniculus* L. C. R. Seances Soc. Biol. Fil. 157:2068-2070.