[CANCER RESEARCH 35, 3089–3093, November 1975]

Diminished Binding of Thyroid-stimulating Hormone in a Transplantable Rat Thyroid Tumor as a Possible Cause of Hormone Unresponsiveness[1]

E. Mandato, M. F. Meldolesi, and V. Macchia

Centro di Endocrinologia ed Oncologia Sperimentale del C.N.R., Istituto di Patologia Generale, Universitá di Napoli, Naples, Italy

SUMMARY

The adenylate cyclase activity and the binding of ^{125}I-labeled thyroid-stimulating hormone (TSH) of normal and tumor rat thyroid plasma membranes were compared. No significant difference in the basal and fluoride-sensitive adenylate cyclase activity between normal and tumor plasma membranes was observed. Thyroid plasma membranes responded to TSH, whereas the enzyme from the tumor plasma membranes was TSH insensitive. Thyroid plasma membranes bound ^{125}I-TSH. Tumor plasma membranes bound ^{125}I-TSH poorly. At the highest concentration of unlabeled TSH used, 80% of the ^{125}I-TSH that was bound to thyroid plasma membranes was displaced, whereas only 10% of the ^{125}I-TSH bound to tumor plasma membranes was displaced. Therefore, it seems likely that the failure of this tumor to respond to TSH is due to an alteration in the functional unit of membrane adenylate cyclase at the level of the receptor subunit.

INTRODUCTION

It is usually believed that the altered behavior of tumor cells is secondary to changes in surface membrane components (4, 5, 15). One of these components is the enzyme adenylate cyclase, which converts ATP to cyclic AMP.[2] Anderson *et al.* (2, 3) have demonstrated decreases in adenylate cyclase activity in virus-transformed fibroblasts, and Klein *et al.* (7) have shown a defect of the adenylate cyclase system in maximally deviated (rapidly growing) Morris hepatomas.

We have previously studied a transplantable rat thyroid tumor and found that its iodine, glucose, and phospholipid metabolism responds to cyclic AMP and not to TSH (11) because adenylate cyclase is unresponsive to TSH (12). The failure of TSH to stimulate the adenylate cyclase of these rat thyroid tumors could be secondary to an alteration in either the TSH receptor, the catalytic subunit of these neoplastic cells, or a factor that couples the receptor by the catalytic subunit.

In order to clarify this point we have studied the binding of ^{125}I-TSH and the adenylate cyclase activity of normal rat thyroid tissue and the transplantable rat thyroid tumor. We found that the tumor has a marked decrease in TSH binding.

MATERIALS AND METHODS

Cyclic AMP, ATP, phosphoenolpyruvate, and pyruvate kinase were purchased from Boehringer/Mannheim, Mannheim, Germany; [α-^{32}P]ATP (0.73 Ci/mmole) was from The Radiochemical Centre, Amersham, England; all other chemicals were of reagent grade. Fischer rats were from Charles River Laboratories (Chicago, Ill.). The thyroid tumor line used throughout these studies was one of a series of thyroid tumors developed in Fischer rats by Wollman (21) and designated as line 1-8 in Wollman's classification. The tumor was kindly supplied by Dr. Wollman (National Cancer Institute, NIH, Bethesda, Md.). All of the tumors used were derived from a single tumor that was carried by s.c. implantation through 7 generations of male Fischer rats during the course of this study. The tumor did not appear to undergo significant alteration in function during this time. The tumors were collected approximately 2 months after s.c. implantation when they had attained an average size of 5 g, and they were kept at 1° until used. Data concerning the histology, the growth rate, and some metabolic properties of this tumor have been previously reported (12). Control thyroids were taken from non-tumor-bearing rats.

Hormone Preparation. Bovine TSH (20 units/mg) was a gift of Dr. Peter Condliffe (NIH, Bethesda, Md.). Labeling of thyrotropin with ^{125}I was performed according to the method of Thorell and Johansson (16) using lactoperoxidase oxidation of iodide in the presence of H_2O_2. The reaction mixture contained 5 mCi of ^{125}I, 0.5 M phosphate buffer (pH 7.4), 36 μg of bovine TSH, 0.26 μmole of H_2O_2, and 25 μg of lactoperoxidase in a final volume of 200 μl. The incubation was carried out for 10 min at room temperature and was stopped by the addition of 500 μl of 0.5 M phosphate buffer. The mixture was then passed through a 1- x 10-cm column of Sephadex G-50 equilibrated with 0.25 M

[1] This work was partly supported by the Italy-United States Cooperation Program in Science (No. 20413154.26368).

[2] The abbreviations used are: cyclic AMP, cyclic adenosine 3':5'-monophosphate; TSH, thyroid-stimulating hormone.

Received April 7, 1975: accepted August 4, 1975.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/35/11/3089/2393663/crs0350113089.pdf by guest on 10 August 2024

Tris-HCl (pH 8.0) and 0.4% bovine serum albumin and eluted with the same buffer. The top fractions of the peak eluting in the void volume representing one-half of the peak radioactivity were pooled and submitted to a gel filtration on a 1- x 10-cm column of Sephadex G-100 equilibrated with 10 mM Tris-HCl (pH 7.4) and 1% bovine serum albumin and eluted with the same buffer. The top fractions of the eluted radioactivity peak were pooled and stored at −80°. The biological activity of the labeled hormone was measured using the glucose oxidation test on hog thyroid slices as previously described (13). The ^{125}I-TSH preparation used retained almost complete biological activity. The specific activity of ^{125}I-TSH was 2 μCi/pmole. The counting efficiency for ^{125}I in the Auto-Gamma counter was 50%.

Purification of Plasma Membranes. Plasma membranes were prepared from normal thyroid and rat thyroid tumors by a modification of the Neville technique, (described in Ref. 22), all procedures being performed at 0–2°. The glands and the tumors that were free of their surrounding tissues were minced and washed in 0.154 M NaCl, and 100 mg of tissue were homogenized in 0.4 ml of ice-cold buffer containing 10 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ (Buffer A), and 0.21 M sucrose with 3 strokes in a Teflon-glass homogenizer. This homogenate was filtered through 2 layers of cheesecloth and was centrifuged at 600 × *g* for 15 min in a Beckman Model J-21B centrifuge. The lipids and the supernatant were removed by aspiration, and the pellet was washed twice by gently resuspending the pellet in the homogenization buffer and centrifuging at the same speed. Then the pellet was resuspended in 0.4 ml of 0.01 M Tris-HCl (pH 7.4), 5 mM $MgCl_2$, and 55% (w/v) sucrose and was brought to a refractive index of 1.435 (57%, w/v) with a saturated solution of sucrose. This suspension was subjected to discontinuous sucrose gradient centrifugation. The gradient consisted of 72% (w/v) sucrose (6 ml), 57% (w/v) sucrose, initial suspension (27 ml), and 24% (w/v) sucrose (5 ml) (refractive indices, 1.470, 1.435, and 1.370, respectively) in 0.01 M Tris-HCl and 0.005 M $MgCl_2$. Centrifugation was carried out in a Spinco Model L-75 ultracentrifuge and an SW 27 rotor for 75 min at 120,000 × *g*. Plasma membranes were harvested from the 24 to 57% interface, were diluted 3-fold with 0.21 M sucrose in 0.010 M Tris-HCl containing 0.005 M $MgCl_2$, and were centrifuged at 11,000 × *g* for 30 min. The final plasma membrane pellet was suspended as concentrated in approximately one-tenth of the initial volume of Buffer A containing 0.21 M sucrose and was stored in small aliquots at −20°. Individual tubes were thawed and diluted to the required protein concentration, prior to use, in the binding and adenylate cyclase assay.

^{125}I-TSH Binding Assay. In the standard assay an aliquot of plasma membranes (50 to 20 μg protein) was incubated at 23° for 10 min except where indicated in the legend, in 150 μl of 20 mM Tris-HCl (pH 7.6), 1 mM EDTA, and 1.3% bovine serum albumin (Buffer B) with 0.22 pmole of ^{125}I-TSH. The reactions were stopped by the addition of 2.5 ml of ice-cold Buffer B and the mixture was filtered through an oxoid filter (1). The filters were presoaked in 10% bovine serum albumin for at least 1 hr and were prewashed with 2 ml of Buffer B before use. The filters with the absorbed membrane:hormone complex were washed twice with 2.5 ml of Buffer B, dried, and counted in an Auto-Gamma counter. All assays were performed in duplicate. Control incubations were performed with all constitutents except membranes. The control values were as low as 2 to 4% of the experimental value.

Adenylate Cyclase Assay. The adenylate cyclase activity was assayed by measuring the conversion of [^{32}P]ATP to cyclic [^{32}P]AMP (8). Each reaction tube contained 0.1 mM ATP, 6 mM $MgCl_2$, 10 mM Tris-HCl (pH 7.4), 5 mM KCl, 10 mM theophylline, pyruvate kinase (70 μg/ml), 2.6 mM phosphoenolpyruvate, and [^{32}P]ATP (2×10^5 cpm). The incubations were started by adding plasma membrane, which had been kept at 1°, to other components, which were at 23°. TSH and other stimulatory substances were added to the homogenates just before the incubations were initiated. The final volume was 0.07 ml. At the end of the incubations at 37°, the reactions were stopped by adding 0.1 ml of solution containing 5 μmoles of ATP, 1.5 μmoles of cyclic AMP, and 0.1 μCi of cyclic [^{3}H]AMP and were boiled for 3 min. To each tube was added 1 ml of 10 mM Tris-HCl buffer, pH 7.8; the contents were stirred on a vortex mixer and the tubes were centrifuged for 10 min at 2500 rpm. The supernatants were applied to columns of alumina prepared as described by White and Zenzer (18) except that the alumina was equilibrated with 10 mM Tris-HCl, pH 7.8. The 1st 3 ml from the column containing cyclic AMP were collected, mixed with 6 ml of Instagel, and counted in a liquid scintillation counter.The cyclic [^{3}H]AMP served to determine the recovery of cyclic AMP during the procedure; recovery ranged from 70 to 80%. Adenylate cyclase was also measured by a chromatographic method. Aliquots of reaction mixture were chromatographed on silica gel plates, with unlabeled ATP, cyclic AMP, ADP, and AMP markers using isopropyl alcohol: water:ammonium hydroxide (7:2:1) (6). The spots were located under UV, scraped from the plates, and counted in Bray's solution. All protein measurements were performed by the procedure of Lowry *et al.* (9) with crystalline bovine serum albumin as standard. Membrane protein was determined after solubilization of appropriate aliquots in 1 N NaOH and heating at 100° for 5 to 30 min.

RESULTS

The yield of adenylate cyclase activity and protein obtained in the course of preparing the plasma membrane fractions from normal rat thyroids and tumors is shown in Table 1. In tumor homogenates the basal level of adenylate cyclase activity is slightly higher than in thyroid homogenates when enzyme activity is related to mg protein. This difference is, however, due to the higher content of thyroglobulin in the normal thyroid homogenate compared to tumor homogenate. No significant difference in the basal level of adenylate cyclase in normal and tumor thyroid plasma membranes is observed. The specific activity of the basal enzyme increased by a factor of about 10 from normal

Downloaded from http://aacrjournals.org/cancerres/article-pdf/35/11/3089/2393663/crs0350113089.pdf by guest on 10 August 2024

Table 1

Yield of adenylate cyclase and protein in preparation of partially purified normal and rat tumor thyroid plasma membranes

Tissue	Substances[a]	Homogenate[b]	Partially purified[b] plasma membranes	Recovery of total activity in membrane (%)
		Adenylate cyclase activity[c] (pmoles cyclic AMP/mg protein/10 min)		
Thyroid	None	0.8	8.8	23
	TSH	1.9	18.0	31
	NaF	16.0	180.0	24
Tumor	None	1.3	10.4	24
	TSH	1.3	10.2	22
	NaF	16.0	200.0	23
		Amount of protein (mg/g, wet wt)		
Thyroid		144.0	2.0	
Tumor		120.0	3.1	

[a] Substance concentration, TSH, 2×10^{-9} M; NaF, 10 mM.

[b] Enzyme concentration, 20 µl of thyroid or tumor fractions (0.7 mg protein).

[c] Substrate concentration: ATP, 100 µM. Incubation was carried out at 37° for 10 min (final pH, 7.5), in the presence of 10 mM Tris-HCl; 5 mM KCl; 10 mM theophylline; 70 µg pyruvate kinase per ml; 2.6 mM phosphoenolpyruvate; and [^{32}P]ATP (2×10^5 cpm).

and about 8 from the tumors as compared with the total homogenates. Thyroid plasma membranes respond to TSH as well as the whole homogenate, whereas the enzyme from the tumors is TSH insensitive in both the whole homogenate and plasma membranes. The ratio of basal to fluoride-stimulated activity is about 1:20 in both normal and tumor thyroid plasma membranes. Except for the hormone-responsive enzyme, whose recovery is 30%, the recovery of the unstimulated and fluoride-sensitive enzyme from both tissues in the plasma membrane fraction was 22 to 24%. The yield of membrane protein in thyroid plasma membranes was 2.0 mg/g of fresh tissue, wet weight, and 3.1 mg in tumor plasma membranes.

The effects of pH and salts concentrations on the ^{125}I-TSH binding to thyroid and tumor plasma membranes were checked. These results are not included in this paper, since they are similar to those previously described by Amir *et al.* (1). However, all binding studies, here reported, were done at the optimum pH and salts concentrations.

At a concentration of 7.5 µg of thyroid membrane protein per reaction mixture 12% of the labeled hormone is bound, whereas at higher protein concentration (22.6 µg/ml reaction mixture) the tumor plasma membranes binds only 2% (Table 2). The binding of TSH to membranes was linearly related to membrane protein concentration; at the highest amount of thyroid membrane protein tested (400 µg/ml), 20% of labeled hormone was bound (data not shown). The amount of labeled TSH bound to tumor plasma membranes, at all protein concentrations used, was much lower than that of thyroid reaching; at the highest protein

Table 2

Binding of ^{125}I-TSH to tumor and thyroid plasma membranes

Membrane preparation	^{125}I-TSH bound[a] (cpm reaction mixture/5 min)		% TSH bound
	− membrane	+ membrane	
Thyroid	710	52,000	11.8
Tumor	430	8,900	2.0

[a] ^{125}I-TSH binding was measured as previously described under "Materials and Methods." Each reaction mixture contained, in 150 µl of 20 mM Tris-HCl (pH 7.6), 1 mM EDTA and 1.3% bovine serum albumin, 0.22 pmole of ^{125}I-TSH (450,000 cpm) and 7.5 µg of protein thyroid plasma membrane, or 22.6 µg of protein tumor membrane. Incubation time, 5 min at 23°.

concentration (400 µg/ml) it reached a maximum of 5% (data not shown). The binding of labeled TSH to thyroid plasma membranes was very rapid, attaining a constant level within 20 min of incubation at either 4° or 23°. The amount of bound TSH was, however, 3-fold greater at 23° than at 4°. At the optimum protein concentration for both thyroid (50 µg/ml) and tumor (150 µg/ml) plasma membranes, there is a 8-fold higher amount of labeled hormone bound to thyroid plasma membranes than to tumor plasma membranes at all incubation times tested (Chart 1). This difference could be significantly increased when the specific TSH bound was considered. The specificity of the binding of ^{125}I-TSH to thyroid plasma membranes has been investigated by measuring the ability of unlabeled TSH to compete for the TSH receptor sites. At a concentration of 2.5×10^{-9} M unlabeled TSH displaced approximately 50% of the ^{125}I-TSH bound to normal thyroid plasma membranes and only 10% of that bound to thyroid tumor plasma membranes (Chart 2). At the highest concentration of unlabeled TSH (10^{-6} M), 80% of the ^{125}I-TSH bound to thyroid plasma membranes was displaced, whereas only 10% of the ^{125}I-TSH bound to tumor plasma membranes was displaced; at 10^{-6} M cold TSH levels, the hormone remaining bound presumably represents nonspecific binding. Therefore, when the specific TSH bound to plasma membranes (considered as the amount of hormone displaced by unlabeled TSH at concentration of 10^{-6} M) was plotted as a function of incubation time, a 10-fold difference in binding TSH appeared (Chart 3). Other proteins such as γ-globulin or albumin added at equivalent unlabeled TSH concentrations did not displace the labeled TSH binding by the tumor membrane preparations.

DISCUSSION

The action of TSH on thyroid tissue, like most of the actions of other polypeptide hormones on their specific target tissues, appears to be mediated at least in part by the adenylate cyclase:cyclic AMP system. The complex interaction of TSH with the adenylate cyclase system in isolated normal thyroid plasma membranes has been intensively studied in the last few years. These studies have provided

Downloaded from http://aacrjournals.org/cancerres/article-pdf/35/11/3089/2393663/crs0350113089.pdf by guest on 10 August 2024

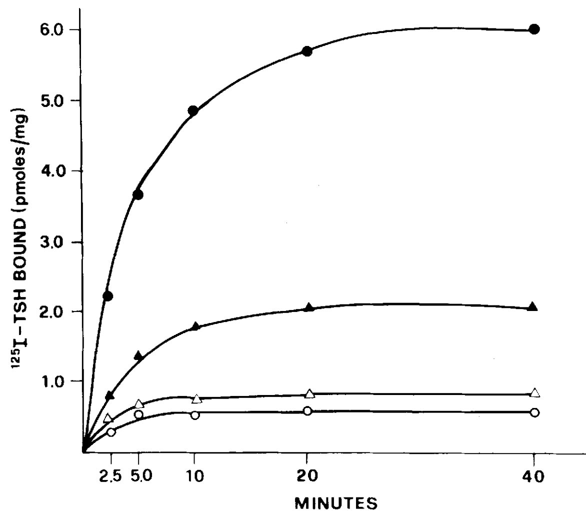

Chart 1. Binding of ^{125}I-TSH to rat thyroid and tumor plasma membranes as a function of time. Experimental conditions are identical to those described in the text. Each reaction mixture contained 7.5 μg of protein thyroid plasma membrane or 22.6 μg of protein tumor membrane. Incubation time, 10 min. Thyroid plasma membranes incubated at 23° (●) and 4° (▲). Tumor plasma membranes incubated at 23° (O) and 4° (Δ).

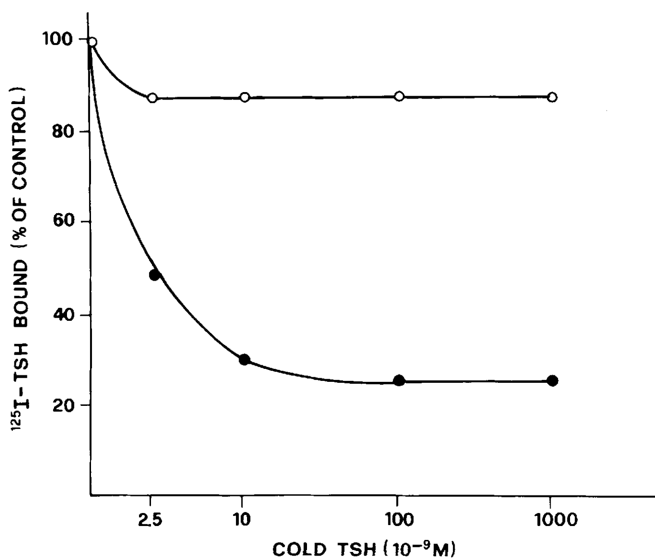

Chart 2. Effect of unlabeled TSH on the binding of ^{125}I-TSH to thyroid and tumor plasma membranes. Experimental conditions are identical to those described in the text except that each reaction mixture contained 5 μg of protein thyroid plasma membrane or 16 μg of protein tumor plasma membranes. Incubation time, 10 min at 23°. Thyroid plasma membranes (●); tumor plasma membranes (O).

information relative to the binding of labeled hormone to specific membrane receptor sites (17, 20), the role of nucleotides in the binding and activation process, and the role of phospholipids in the activation process (19). Not very much is known about the modification of the adenylate cyclase:cyclic AMP system in states of altered thyroid function. We have previously reported that a transplantable rat thyroid tumor has lost the ability to respond to TSH

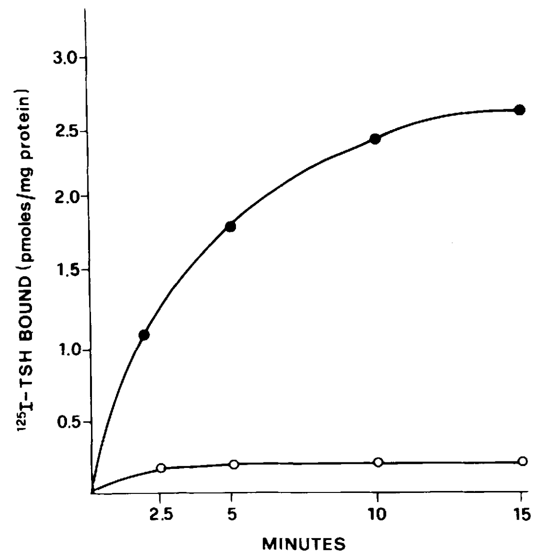

Chart 3. Specific binding of ^{125}I-TSH to rat thyroid and tumor plasma membranes as a function of incubation time. Experimental conditions are identical to those of Chart 2.

(11). More recently, we demonstrated that the obliteration of the TSH response in this tumor is not due to the inability of the cell to respond to cyclic AMP but to the inability of TSH to activate adenylate cyclase (12).

The TSH-responsive adenylate cyclase is a complex enzyme. It consists of a catalytic site and TSH-binding site, has a requirement for phospholipid, and probably contains other unidentified components. Evidence for the presence of other components comes from the observation that binding of the hormone to the receptor site can be dissociated from activation of adenylate cyclase (14), since treatment with a specific phospholipase C of thyroid slices does not interfere with the binding of TSH to the thyroid plasma membranes, whereas this treatment abolishes the activation of adenylate cyclase by TSH (10). Furthermore, other authors have shown that phospholipase A treatment abolishes the response of adenylate cyclase to TSH; however, when plasma membranes were incubated with phospholipase A, subsequent addition of phosphatidylcholine but not phosphatidylethanolamine partially restored TSH stimulation.

In this paper we have analyzed the loss of TSH response of rat thyroid tumor in terms of the current model of the membrane adenylate cyclase. Since no significative difference in the basal and fluoride-sensitive adenylate cyclase between normal and tumor plasma membranes was observed, the catalytic subunit of adenylate cyclase appeared to be intact in this tumor. Thus it seems likely that the defect resides either in the composition of the phospholipids or in the TSH-binding site. The results on the comparison of bindings of TSH to normal and tumor plasma membranes show a significant difference. Tumor plasma membranes bound a significantly lower amount of labeled hormone than did normal thyroid plasma membranes (Chart 1). Furthermore, this difference appears higher when nonspecific binding was taken into account. In fact, unlabeled TSH can displace about 80 to 90% of labeled TSH bound to thyroid

Downloaded from http://aacrjournals.org/cancerres/article-pdf/35/11/3089/2393663/crs0350113089.pdf by guest on 10 August 2024

plasma membranes, whereas from the lowest to the highest unlabeled hormone concentrations only 15% of the labeled TSH bound to the tumor plasma membranes was displaced (Chart 2).

Thus it seems likely that the failure of this tumor to respond to TSH is due at least in part to an alteration in the functional unit of membrane adenylate cyclase at the level of the receptor subunit. From the present data it is, however, not possible to establish whether the failure to bind TSH is due to a physical absence of TSH-binding proteins or to a marked reduction in affinity. Other aspects of the adenylate cyclase of this tumor are currently under study.

ACKNOWLEDGMENTS

The authors thank Dr. I. Pastan, National Cancer Institute, NIH, and Dr. L. D. Kohn, National Institute of Arthritis and Metabolic Disease, NIH, Bethesda, Md., for valuable criticism in reading the manuscript.

REFERENCES

1. Amir, S. M., Carraway, T. F., Jr., Kohn, L. D., and Winand, R. I. The Binding of Thyrotropin to Isolated Bovine Thyroid Plasma Membranes. J. Biol. Chem., *248:* 4092-4100, 1973.
2. Anderson, W. B., Johnson, G. S., and Pastan, I. Transformation of Chick-Fibroblasts by Wild-type and Temperature-sensitive Rous Sarcoma Virus Alters Adenylate Cyclase Activity. Proc. Natl. Acad. Sci. U. S., *70:* 1055-1059, 1973.
3. Anderson, W. B., Lovelace, E., and Pastan, I. Adenylate Cyclase Activity is Decreased in Chick-Embryo Fibroblasts Transformed by Wild-Type and Temperature-sensitive Schmidt-Ruppin Rous Sarcoma Virus. Biochem. Biophys. Res. Commun., *52:* 1293-1299, 1973.
4. Emmelot, P. Biochemical Properties of Normal and Neoplastic Cell Surfaces: A Review. European J. Cancer, *9:* 319-333, 1973.
5. Emmelot, P., and Bas, C. I. Studies on Plasma Membranes. Adenyl Cyclase in Plasma Membranes Isolated from Rat and Mouse Livers and Hepatomas, and Its Hormone Sensitivity. Biochim. Biophys. Acta, *249:* 285-292, 1971.
6. Giannattasio, M., Sica, G., and Macchia, V. Cyclic AMP Phosphodiesterase from Dormant Tubers of Jerusalem Artichoke. Phytochemistry, *13:* 2729-2733, 1974.
7. Klein, I., Levely, G. S., Bricker, L. A., and Morris, H. P. Glucagon and Epinephrine Activation of Adenylate Cyclase and Glucagon Binding in Morris Hepatomas. Endocrinology, *94:* 279-283, 1974.
8. Krishna, G. B. Weiss, B., and Brodie, B. B. A Simple, Sensitive Method for the Assay of Adenyl Cyclase. J. Pharmacol. Exptl. Therap., *163:* 379-385, 1968.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem., *193:* 265-275, 1951.
10. Macchia, V., and Meldolesi, M. F. Cell Membrane of Thyroid and Its Altered Responsiveness to the Hormone TSH. Advan. Cytopharmacol., *2:* 33-37, 1974.
11. Macchia, V., Meldolesi, M. F., and Chiariello, M. Effect of TSH and TSH-like Substances on Some Properties of a Transplantable Thyroid Tumor of the Rat. *In:* K. Fellinger and R. Hofer (eds.), Further Advances in Thyroid Research, pp. 1205-1213. Vienna: Verlag der Wiener Medizinischen Akademie, 1971.
12. Macchia, V., Meldolesi, M. F., and Chiariello, M. Adenyl-Cyclase in a Transplantable Thyroid Tumor: Loss of Ability to Respond to TSH. Endocrinology, *90:* 1483-1491, 1972.
13. Macchia, V., Tamburrini, O., and Pastan, I. Role of Lecithin in the Mechanism of TSH Action. Endocrinology, *86:* 787-792, 1970.
14. Macchia, V., and Varrone, S. "Thyroid Cell Membrane and Mechanism of TSH-Action". *In:* Proceedings of the Fourth International Congress of Endocrinology, pp. 539-542. Amsterdam: Excerpta Medica, 1972.
15. Perdue, J. F., Warner, D., and Miller, K. The Isolation and Characterization of Plasma Membrane from Culture Cells. The Chemical Composition of Plasma Membranes Isolated from Chicken Tumors Initiated with Virus-Transformed Cells. Biochim. Biophys. Acta, *298:* 817-826, 1973.
16. Thorell, J. I., and Johansson, B. G. Enzymatic Iodination of Polypeptides with ^{125}I to High Specific Activity Biochim. Biophys. Acta, *251:* 363-369, 1971.
17. Verrier, B., Fayet, G., and Lissitzky, S. Thyrotropin Binding Properties of Isolated Thyroid Cells and Their Purified Plasma Membranes. European J. Biochem., *42:* 355-365, 1974.
18. White, A. A., and Zenzer, T. V. Separation of Cyclic 3',5'-Nucleoside Monophosphates from Other Nucleotides on Aluminum Oxide Columns. Application to the Assay of Adenyl Cyclase and Guanyl Cyclase. Anal. Biochem., *11:* 372-396, 1971.
19. Wolff, J., and Cook, G. H. Activation of Thyroid Membrane Adenylate Cyclase by Purine Nucleotides. J. Biol. Chem., *248:* 350-355, 1973.
20. Wolff, J., and Jones, A. B. The Purification of Bovine Thyroid Plasma Membranes and the Properties of Membrane-bound Adenyl Cyclase. J. Biol. Chem., *246:* 3939-3947, 1971.
21. Wollmann, S. H. Effects of Feeding Thiouracil on Thyroid Glands of Rats. J. Natl. Cancer Inst., *26:* 473-485, 1961.
22. Yamashita, K., and Field, J. B. Isolation of Plasma Membrane from the Thyroid. Methods Enzymol., *31A:* 144-149, 1974.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/35/11/3089/2393663/crs0350113089.pdf by guest on 10 August 2024