PREPARATION OF THYROID PLASMA MEMBRANES CONTAINING A TSH-RESPONSIVE ADENYL CYCLASE

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SUMMARY

Adenyl cyclase activity was assayed in a plasma membrane fraction prepared from bovine thyroid using sucrose gradients. Its specific activity was ten times greater than that of the original homogenate. The activity in the plasma membrane fraction was stimulated five-fold by TSH as compared to only a two-fold increase in the whole homogenate. Electron microscopy of the membrane fraction indicated that it was contaminated by only a minimum amount of other cellular components. The Na^+-K^+-activated ATPase in this fraction had a specific activity ten times that in the original homogenate. The membrane fraction was devoid of cytochrome C oxidase but did contain higher specific activity DPNH-cytochrome C reductase than the original homogenate.

The adenyl cyclase - cyclic AMP system appears to mediate the effects of many hormones on their respective target tissues (1). Recent evidence indicates that TSH regulates thyroid gland function by this mechanism. TSH stimulated adenyl cyclase activity in washed particulate preparations obtained from sheep thyroid (2) and in beef and dog thyroid homogenates (3, 4). It also rapidly increased the concentration of cyclic AMP in bovine (5) and canine (6) thyroid slices.

Although adenyl cyclase is probably associated with the plasma membrane of the cell (7, 8), Stanbury et al (9) were unable to detect the enzyme in plasma membranes obtained from human and bovine thyroids. Enzyme studies and electron microscopy of their preparation confirmed the presence primarily of plasma membranes. Furthermore, Burke (10) reported maximum adenyl cyclase activity was obtained in the mitochondrial and microsomal fractions obtained from sheep thyroid homogenates separated by the technique of DeGroot and

Carvalho (11). Since these fractions also contained 5'-nucleotidase he did not exclude the possibility that they were contaminated with plasma membrane fragments. Butcher and Serif (12) reported the highest specific activity of adenyl cyclase in dog thyroid was found in the 600 x g fraction while maximum responses to TSH were obtained with particulate preparations obtained at 9,000 x g. In order to localize the adenyl cyclase of the thyroid, thyroid plasma membranes were prepared and tested for their responsiveness to TSH. While these studies were in progress, Wolff et al reported preliminary results indicating TSH stimulation of adenyl cyclase in a plasma membrane fraction prepared from beef thyroid (13). They did not provide details concerning their method of preparation or the purity of their preparation.

MATERIALS AND METHODS

Bovine thyroids, obtained from a local abattoir, were transported to the laboratory on ice. Plasma membranes were isolated by the method of Neville (14) as modified by Emmelot et al (15). All operations were done at 0-4° C. Six grams of minced thyroid tissue were homogenized in 50 ml of 1 mM $NaHCO_3$ (pH 7.5) in a loose-fitting Dounce homogenizer. The homogenate was diluted to 200 ml using 1 mM $NaHCO_3$ and kept on ice for several minutes. After filtering the homogenate through four layers of numbers 40 and 120 cheesecloth, it was centrifuged at 2,600 rpm for 25 minutes. The precipitate was resuspended in 30 ml of the $NaHCO_3$ solution and centrifuged at 2,600 rpm for 15 minutes. The precipitate was suspended in 4 ml of 1 mM $NaHCO_3$, mixed with 10 ml of 63% sucrose, and the density of the suspension adjusted to 1.22. Sucrose solutions of density 1.20 (7 ml), 1.18 (10 ml) and 1.16 (2 ml) were carefully layered over it. The tube was centrifuged at 25,000 rpm for 120 minutes using a SW 25 rotor. After centrifugation there was material at the interface between the layers of density 1.16 and 1.18 and a pellet at the bottom of the tube. These were collected separately, diluted 1:1 with 1 mM $NaHCO_3$ and centrifuged at 16,000 rpm for 30 minutes

in a Sorvall centrifuge. The precipitates were suspended in 0.25 ml of 1 mM $NaHCO_3$ for enzyme assays and electron microscopy.

The preparations were assayed for the following enzymes: adenyl cyclase (4), cytochrome C oxidase (16), DPNH-cytochrome C reductase (17) and ATPase (9). Protein was measured by the method of Lowry et al (18). TSH was kindly provided by the Endocrinology Study Section, National Institutes of Health.

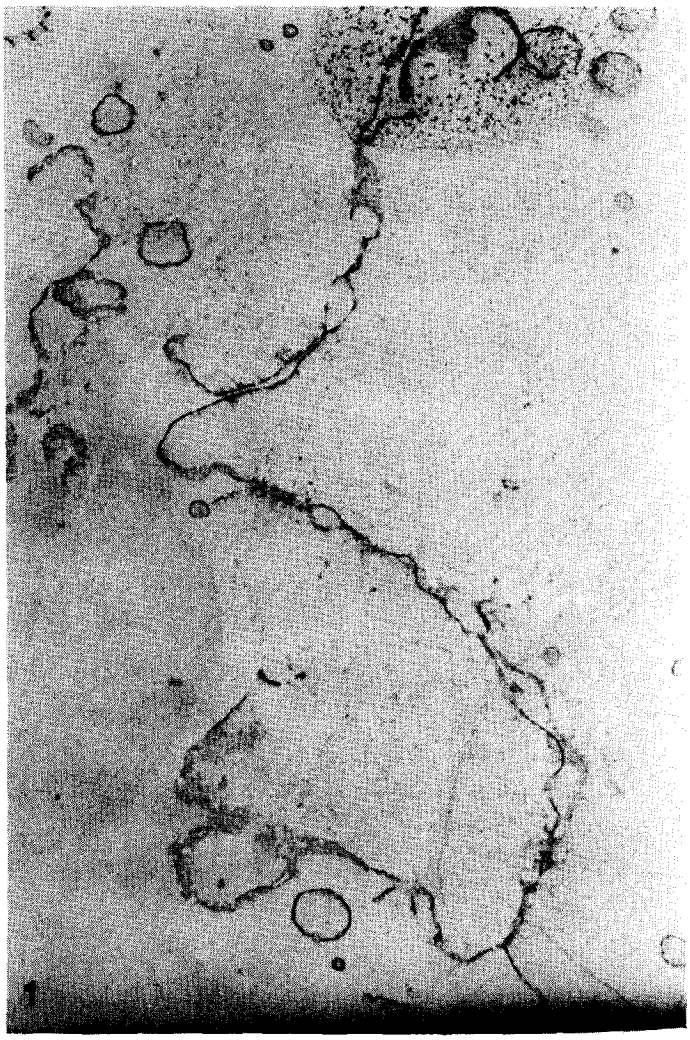

Figure 1. Electron micrograph of material obtained at interface of sucrose layers of density 1.16 and 1.18. Most of the structures represent membranes with occasional desmosomes. Magnification was 24,800 x.

RESULTS

Figure 1 is an electron micrograph of the material at the interface between the layers of density 1.16 and 1.18. The main structures shown consist of vesicular membranous material and a continuous layer of plasma membranes with a minimum amount of other cellular components, primarily rough reticulum. The material in Figure 2 is representative of the pellet at the bottom of the tube. It consists primarily of amorphous debris, but also contains some nuclei, rough reticulum, mitochondrial fragments and plasma membranes.

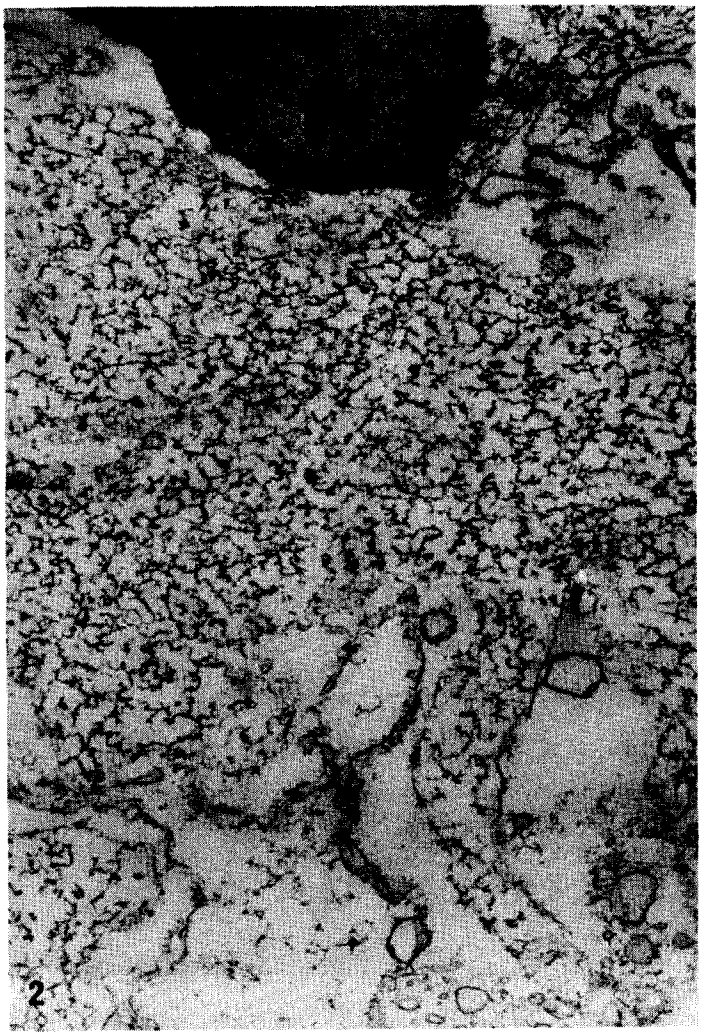

Figure 2. Electron micrograph of pellet (sectioned) illustrating presence of nuclear material, rough reticulum, smooth reticulum, and coagulated protein. Although not illustrated, there were also occasional mitochondria in the pellet. Magnification was 24,800 x.

TABLE 1

Effects of TSH and NaF on adenyl cyclase activity in whole homogenate, plasma membrane fraction and pellet

		Experiment 1	Experiment 2
		cpm/mg protein	cpm/mg protein
Whole homogenate	Control	184	124
	TSH 10 mU	331	254
	NaF 10^{-2} M	1,259	1,527
Plasma membrane fraction	Control	1,826	2,108
	TSH 10 mU	10,246	10,747
	NaF 10^{-2} M	46,759	61,729
Pellet	Control	0	210
	TSH 10 mU	48	293
	NaF 10^{-2} M	1,315	3,321

The results are the average of duplicate determinations. 10 mU of TSH was added to the appropriate tube. The final concentration of NaF was 10^{-2} M.

The data in Table 1 summarize the adenyl cyclase activity in the whole homogenate, the plasma membrane fraction and in the pellet from the bottom of the tube. The specific activity of the adenyl cyclase activity in the plasma membrane fraction was 10-20 times that in the whole homogenate. Furthermore, TSH increased the activity five-fold in the plasma membrane fraction as compared to only two-fold in the whole homogenate. In contrast very little adenyl cyclase activity was demonstrable in the pellet and effects of TSH were either minimal or absent. NaF stimulated the enzyme in all three preparations but the effect was also greatest in the plasma membrane fractions.

The cytochrome C oxidase, DPNH-cytochrome C reductase and ATPase content of the whole homogenate, plasma membrane fraction and pellet are summarized in Table 2. Although cytochrome C oxidase was present in the whole homogenate, it was not detected in either the plasma membrane fraction or the pellet. ATPase was present in all three fractions but had ten times the specific activity

TABLE 2

Cytochrome C oxidase, DPNH-cytochrome C reductase, and ATPase activities in whole homogenate, plasma membrane and pellet

	cytochrome C oxidase	DPNH-cytochrome C reductase	ATPase
Whole homogenate	0.012*	0.18*	0.026**
Plasma membrane fraction	0	1.19	0.27
Pellet	0	1.04	0.058

* mumoles/min/mg protein

** mg PO_4/hr/mg protein

The results are the average of duplicate determinations.

in the plasma membrane fraction as compared to the original homogenate. This represents approximately the same amount of purification as obtained when adenyl cyclase was assayed. The pellet contained ATPase of twice the specific activity as that found in the whole homogenate. DPNH-cytochrome C reductase had a six-fold increase in its specific activity in the plasma membrane fraction compared to the whole homogenate. The specific activity in the pellet was approximately equivalent to that in the plasma membrane fraction.

DISCUSSION

Electron microscopic examination of the material at the interface of the layers of density 1.16 and 1.18 confirmed the presence of plasma membranes, with only minor contamination by other cellular components. The ten-fold increase in the specific activity of the Na^+-K^+-activated ATPase in this fraction is further evidence that it contained plasma membranes. The absence of cytochrome C oxidase indicates very little, if any, contamination with mitochondria. DPNH-cytochrome C reductase was concentrated six-fold in the

membrane fraction indicating the presence of some microsomal elements. Emmelot et al (15) reported similar findings in their preparations of liver plasma membranes. Although morphologically it was difficult to identify all of the material in the pellet, it contained about as much DPNH-cytochrome C reductase as did the membrane fraction.

The demonstration of adenyl cyclase activity in the plasma membrane fraction is consistent with the previous reports that the enzyme is associated with this fraction in pigeon erythrocytes (7) and rat liver (8). The failure of Stanbury et al (9) to detect adenyl cyclase activity in plasma membrane fractions prepared from bovine and human thyroids is unexplained. They utilized nitrogen under pressure for the preparation of their membrane fragments and obtained a fraction that had enzyme activities usually attributed to the plasma membrane. Their results suggest that adenyl cyclase activity may be more labile than some of the other enzymes associated with the plasma membrane. Storage of the membrane fraction overnight at 4° C caused about a 30% decrease in both the basal and TSH-stimulated adenyl cyclase activity. Burke (10) and Butcher and Serif (12) reported thyroid adenyl cyclase activity in the 9,000 x g particulate fraction responded maximally to TSH. However, since these fractions contained significant amounts of 5'-nucleotidase, contamination with plasma membrane fragments is almost certain. Our preparation is more purified than these and was more responsive to TSH than was the whole homogenate. Butcher and Serif also found that their 9,000 x g fraction was more responsive to TSH than the whole homogenate (12). The explanation for this is not apparent but could indicate removal of an inhibitor of TSH stimulation during the process of purification of the membrane fraction. This seems unlikely however, since the stimulation of adenyl cyclase in the membrane fraction by NaF was also greater than that observed in whole homogenate. As previously reported NaF is a more potent stimulator of adenyl cyclase in the thyroid than is TSH, but the physiologic significance of such stimulation is not known (4).

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