ISOLATION OF PLASMA MEMBRANES FROM BOVINE CORPUS LUTEUM POSSESSING ADENYLATE CYCLASE, ^{125}I-hCG BINDING AND Na-K-ATPase ACTIVITIES*.

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SUMMARY

Plasma membranes from bovine corpora lutea have been purified by sucrose density gradient centrifugation. The purified membranes, in addition to binding ^{125}I-hCG, also possess hCG-stimulated adenylate cyclase and Na-K-ATPase. The relative purification of ^{125}I-hCG binding, adenylate cyclase and Na-K-ATPase on the basis of the specific activities in the whole homogenate were 7.8, 6.4 and 2.6, respectively. The presence of both the hormone sensitive adenylate cyclase and ^{125}I-hCG binding activities suggest that these plasma membranes might possess the 'receptor' for gonadotropin.

Adenosine 3',5'-cyclic monophosphate has been shown to play an intermediatary role in LH* mediated steroidogenesis in the bovine corpus luteum (1). Although LH is also directly capable of stimulating some of the intracellular events in bovine corpus luteum (2), it is believed that one of the primary events of LH action is to stimulate the plasma membrane bound adenylate cyclase as has been described with other protein or polypeptide hormones in their respective target tissues (3). In this communication, we describe the isolation of plasma membranes from bovine corpus luteum which possess 1) hormone stimulated

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*The following abbreviations are used: LH, luteinizing hormone; hCG, human chorionic gonadotropin.

adenylate cyclase, 2) the ability to bind ^{125}I-hCG, and 3) sodium and potassium stimulated ATPase.

MATERIALS AND METHODS

Chemicals: Uniformly labeled ^{14}C-ATP, was purchased from New England Nuclear Corporation. Phosphoenolypyruvate and pyruvate kinase were purchased from Sigma Chemical Co. All other chemicals were conventional commercial products.

Assay of Adenylate Cyclase: Adenylate cyclase was assayed by the procedure of Drummond and Duncan (4). This involves incubation of ^{14}C-(U) ATP in the presence of 20 mM phosphoenolypyruvate, 2 units of pyruvate kinase, 6 mM KCl, 15 mM $MgSO_4$, 8 mM theophylline, 2 mM cyclic AMP and 40 mM Tris HCl, pH 7.5. The final volume of the incubation mixture was 150 µl. After terminating the incubation by boiling for 3 min, the solution was centrifuged and 100 µl of the supernatant was chromatographed on paper in 1 M ammonium acetate pH 7.4: Ethanol (3:7) system for 16 hr. After drying the chromatogram, the area corresponding to cyclic AMP was cut and radioactivity was determined by liquid scintillation counting.

Isolation of Plasma Membranes: The procedure used for the isolation of plasma membrane was similar to that described by Marx et. al. (5) for the preparation of plasma membrane fraction from the rat kidney. Bovine corpora lutea (50 gm) were homogenized in 3 vol of Buffer A (0.25 M sucrose, 0.01 M Tris, HCl, pH 7.5 and 0.001 M EDTA), and filtered through 2 layers of cheese cloth to obtain the whole homogenate (WH). The whole homogenate was

then diluted with an equal volume of Buffer A and centrifuged at 4500 rpm in a Sorvall fitted with an SS 34 rotor. When the rotor reached 4500 rpm the motor was turned off. The nuclei and unbroken cells were rejected and the above procedure was repeated twice more with the supernatant. The resulting supernatant was then centrifuged at 4500 rpm for 15 min when a double layered pellet was obtained. The upper portion was resuspended in 2 volumes of Buffer A and centrifuged at 4500 rpm for 15 min. The resulting pellet was resuspended in 6% of the original volume to obtain partially purified membrane (PPM). This fraction was then layered on top of 32-42 percent sucrose gradient and centrifuged at 23,000 rpm for 90 min using an SW 25-1 rotor. After the centrifugation, three distinct layers appeared in the gradient; a top layer of lipids, a middle layer of plasma membranes and a bottom layer of a thick pellet. The middle layer containing the plasma membranes (PM) was then separated.

Preparation of ^{125}I-hCG: HCG was iodinated using chloramine-T method of Hunter and Greenwood (6) with minor modifications (7).

Assay of Na^+ -K^+ ATPase: Na^+ -K^+ -ATPase was assayed by the method of Hendler et. al. (8) by measuring the inorganic phosphate released from ATP in the presence of Na^+ and K^+ minus the inorganic phosphate liberated in the absence of these ions. The inorganic phosphate released was measured by the prodedure of Ames (9).

RESULTS

The specific activities of Na^+ -K^+ -ATPase, adenylate cyclase and the binding of ^{125}I-hCG in the whole homogenate, partially

TABLE I

Summary of the purification of adenylate cyclase ^{125}I-hCG binding activity and Na^+, K^+, ATPase

Treatment	Adenylate cyclase[a]	^{125}I-hCG binding[b]	Na^+, K^+ ATPase[c]
Whole homogenate	359 units	17, 813	341 units
Partially purified membrane	764 units	42, 694	517 units
Purified membrane	2,277 units	139, 621	885 units

[a]One activity unit of adenylate cyclase is 1 picomole of ^{14}C-cyclic AMP formed per mg protein per 10 min at 37°. The assays were performed in the presence of fluoride ion.
[b]CPM of ^{125}I-hCG bound per mg protein per 30 min of incubation at 37° which can be displaced by the addition of unlabelled hCG.
[c]One unit is defined when the net-difference between the inorganic phosphate liberated from ATP in the presence and absence of Na^+, K^+ and Mg^{2+} is equal to 1 nanomole per 10 min at 37°.

purified membranes and purified membranes are shown in Table 1. The specific activities of adenylate cyclase and the binding of ^{125}I-hCG were increased 6.4 and 7.8 fold, respectively. The specific activity of Na^+ -K^+ -ATPase was increased 2.6 fold, as compared to the specific activity in the whole homogenate. When fractions of the sucrose gradient were analyzed for adenylate cyclase activity, the enzyme was present in detectable levels only in the band corresponding to the plasma membranes (middle band).

^{125}I-hCG Binding Reaction: The binding of ^{125}I-hCG to whole homogenate, partially purified membrane and purified membrane preparations are shown in Fig 1. The crossed bars at the bottom

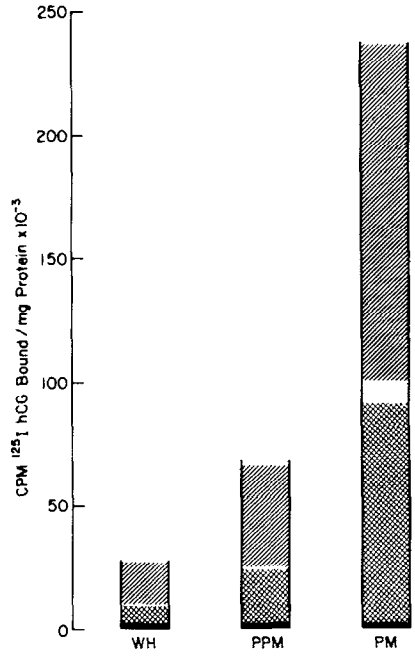

Figure 1 The binding of ^{125}I-hCG to whole homogenate (WH), partially purified membrane (PPM) and purified plasma membrane fractions from bovine corpus luteum. The upper portions of the bars with parallel lines represent ^{125}I-hCG bound which could be competed by unlabelled hCG and the bottom portions of the bars with crossed lines indicate counts bound irreversibly. The solid bars indicate the background radioactivity obtained in duplicate determinations.

indicate non-reversibly bound radioactivity and the top of the bar with parallel lines show the binding of ^{125}I-hCG which was 'reversed' by the addition of an excess amount of unlabelled hCG. The bottom line indicates the non-specific adsorption of radioactivity to glass tubes. The experiments were performed in duplicates which are represented by the dotted lines.

The time course of the binding of ^{125}I-hCG to partially purified plasma membranes is shown in Fig 2. The binding re-

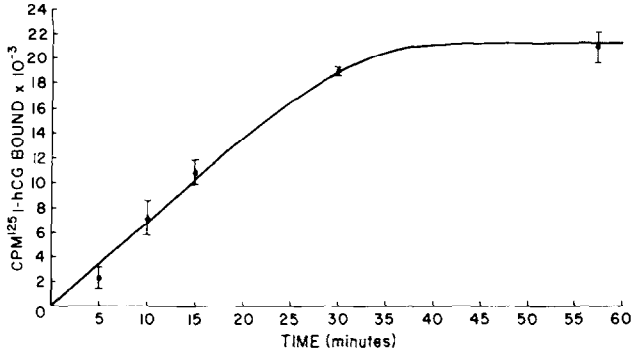

Figure 2 The time course of the binding of ^{125}I-hCG to WH, PPM and PM from bovine corpus luteum. One picomole of ^{125}I-hCG was added to the reaction mixture in control experiment to test the reversibility of the reaction. The data represent the binding reaction which can be displaced by unlabelled hCG as a function of incubation time.

action was linear at 37° up to 15 min of incubation, after which the extent of binding reached a plateau.

Stimulation of Adenylate Cyclase Activity by hCG: The purified membrane fractions also possessed adenylate cyclase activity which was stimulated by the addition of 50 ng of unlabelled hCG. (Table 2). The incubations were performed in the absence of fluoride ion. The data also show that the extent of stimulation caused by hCG was higher in the purified membrane fraction as compared to the stimulation with whole homogenate and the partially purified membranes.

DISCUSSION

The present study demonstrate the partial purification of plasma membranes from the bovine corpus luteum which possess the ability to bind ^{125}I-hCG, hormone sensitive adenylate cyclase

TABLE II

In vitro effect of hCG on the adenylate cyclase activity.

Preparation	hCG added	Enzyme activity
Whole homogenate	0	55
Whole homogenate	50 ng	74
Partially purified membrane	0	124
Partially purified membrane	50 ng	174
Purified membrane	0	323
Purified membrane	50 ng	977

Enzyme activity is expressed as the picomoles of cyclic AMP formed per 30 min per mg of protein. The incubations were performed in the absence of fluoride ion.

and ATPase activity stimulated by Na^+ and K^+. The latter is thought to be involved in the exchange of sodium and potassium ions and thus is widely used as a marker for plasma membranes (10). The parallelism between the extent of purification of the adenylate cyclase activity and the ability to bind ^{125}I-hCG in the purified plasma membranes suggests that these membranes possess the 'receptor' for gonadotropin. The present study do not, however, rule out the existence of other cellular sites of hCG binding in the bovine corpus luteum.

The binding of ^{125}I-hCG to plasma membranes were reversed

by the addition of unlabelled hCG. The reversibility was observed only when the labelled and unlabelled hCG were coincubated with the plasma membranes. A fraction of the radioactivity remained bound to the plasma membranes after the addition of 1 picomole of hCG suggesting the existence of a non-reversible binding component.

The existence of high affinity gonadotropin binding protein has been previously reported in the rat testes (11, 12), ovary (13-15) and corpus luteum (16). The presence of gonadotropin stimulated adenylate has been reported in the low speed sediment of bovine corpus luteum (17). The present experiments demonstrate that in addition to gonadotropin binding protein, the plasma membranes from bovine corpus luteum also possess hormone responsive adenylate cyclase system. Isolation of such a receptor would facilitate attempts to understand the nature of the relationship between the hormone binding protein and the adenylate cyclase - cAMP system.

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