THE ENRICHMENT OF ADENYLATE CYCLASE IN THE

PLASMA MEMBRANE AND GOLGI SUBCELLULAR FRACTIONS

OF PORCINE ADENOHYPOPHYSIS

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INTRODUCTION

Adenylate cyclase has been found to be associated with particulate fractions from various mammalian cells and unicellar organisms (for review see reference 16). Mostly, the enzyme has been associated with the plasma membrane, although in brain it was contained in the synaptic membranes (3), in the rabbit psoas muscle in the microsomal fraction (15), and in the lymphocyte on the plasma and nuclear membranes (17). Although hypothalamic releasing factors seem to stimulate the secretion of certain hormones from adenohypophysis through adenylate cyclase and cyclic AMP (16), the subcellular distribution of the enzyme has not been studied.

This preliminary communication reports the finding of marked enrichment of adenylate cyclase in the plasma membrane and Golgi fractions of porcine adenohypophysis. These subcellular fractions were each enriched in their respective components but cross-contamination did exist as shown by electron microscope and enzyme marker studies.

MATERIALS AND METHODS

Preparation of Fractions

The experimental protocol for preparing each of the subcellular fractions from porcine adenohypophysis has been described in detail previously (8). The fractions were made from pituitaries obtained from a local slaughter house the day of the experiments.

Enzyme Assays and Chemical Procedures

Protein was measured by the method of Lowry et al. (13) on 1 N NaOH extracts of each fraction and compared to fresh standards from stock bovine serum albumin fraction V. Galactosyl transferase was assayed by the method of Babad and Hassid (1) as modified by Lowenstein et al. (12) using N-acetylglucosamine as acceptor. Succinate-cytochrome c reductase and NADH-cytochrome c reductase were measured as described by Fleischer et al. (7). 5'-Nucleotidase was assayed by measuring the release of organic phosphate from 5'-AMP as previously described (14). Adenylate cyclase activity was determined by the method of Krishna et al. (11) as modified by Jarett et al. (9). The enzyme reaction was incubated for 20 min after it was determined that linearity was maintained for at least 30 min. ATPase was assayed as follows: The basic medium for determining

Mg^{++}-ATPase contained 0.05 M Tris-HCl, pH 7.4, 3.5 mM ATP, 3.5 mM Mg SO_4, 0.25 M sucrose, and 50–200 μg of tissue protein in a final volume of 1.0 ml. The reactions were stopped after 30 min at 37°C by the addition of cold perchloric acid. The extracts were neutralized with potassium bicarbonate and inorganic phosphate was determined by the method of Fiske and SubbaRow (5). The medium was modified to contain 118 mM NaCl and 5 mM KCl in place of sucrose to measure the Na^+, K^+-ATPase. RNA was determined by a procedure described by Fleck and Munro (6).

Electron Microscopy

The plasma membrane pellet was initially fixed in 3% glutaraldehyde, 0.1 M cacodylate-HCl, pH 7.4, washed in buffer, postosmicated, stained in 0.5% uranyl acetate in saline, dehydrated, and embedded in Epon 812. The Golgi fraction was processed according to a special procedure of Kanaseki and Kadota (10). Ultrathin sections were stained with alcoholic uranyl acetate and lead citrate and photographed with a Philips EM 200 electron microscope. Fresh plasma membrane or Golgi membrane fractions were negatively stained with 1% phosphotungstic acid before photography.

Materials were obtained from standard commercial sources.

RESULTS

Morphology

The plasma membrane fraction at low magnification (Fig. 1) contained primarily intact vesicles of 0.2–1.5 μm in diameter which contained cytoplasmic remnants. Microfilaments were seen just beneath and attached to the membrane vesicles and were similar to the filaments found adjacent to the plasma membrane of intact porcine anterior pituitary cells, providing morphological evidence for the origin of the vesicles. Higher magnification revealed that some vesicles contained mitochondria, rough endoplasmic reticulum, and free ribosomes. Very few, if any, club-shaped or vesicular profiles characteristic of the Golgi preparation were seen in either thin-sectioned material or negatively stained preparations. Rare secretory granules were the major identifiable extravesicular contaminant.

The Golgi fraction contained mostly structures similar to in situ Golgi apparatuses which were numerous flattened lamellae with terminal expansions and dense luminal contents and dilated cisternae (Fig. 2). The main contaminants were membrane vesicles and secretory granules in vari-

ous stages of development. Negatively stained Golgi preparations showed complex arrays of smooth opaque vesicles, tubules, and club-shaped forms. Occasionally seen were collapsed filmy vesicles similar to those which comprised the negatively stained plasma membrane fraction.

The mitochondria were only moderately well preserved as seen in the plasma membrane fraction. This fraction was morphologically the purest fraction used in this study, although occasionally secretory granules and rough endoplasmic reticulum were seen. The microsomal fraction consisted primarily of small membranous vesicles of both rough and smooth endoplasmic reticulum nature as seen in the intact cell. These vesicles contained the slightly dense amorphous material classically seen in in situ endoplasmic reticulum. Some of the smooth vesicles could be of plasma membrane origin.

Enzyme Markers

The data are summarized in Table I and are presented from a large number of preparations rather than being selected results from single preparations.

The average 5'-nucleotidase activity was enriched over the homogenate 16-fold in the plasma membrane fraction, 10-fold in the Golgi fraction, and sevenfold in the microsomal fraction. This content, in the latter two fractions, of a predominately plasma membrane enzyme is in part related to the small amount of plasma membrane contamination seen by electron microscopy, but also may reflect an activity which is intrinsic to all of these structurally and functionally related membrane systems (2, 4).

The Mg^{++}-ATPase activity was enriched threefold in the plasma membrane fraction, 2.5 times in the microsomes, and less than twice in the Golgi and mitochondria fractions. The Na^+, K^+-ATPase was concentrated almost 3.5-fold in the plasma membrane, while 2.5-fold in the Golgi fraction, and doubled in the microsomal fraction.

Galactosyl transferase, a known marker for liver Golgi fractions (7), was enriched eightfold in the Golgi fraction, tripled in the mitochondrial fraction, doubled in the microsomal fraction, and only slightly increased over the homogenate in the plasma membrane fraction.

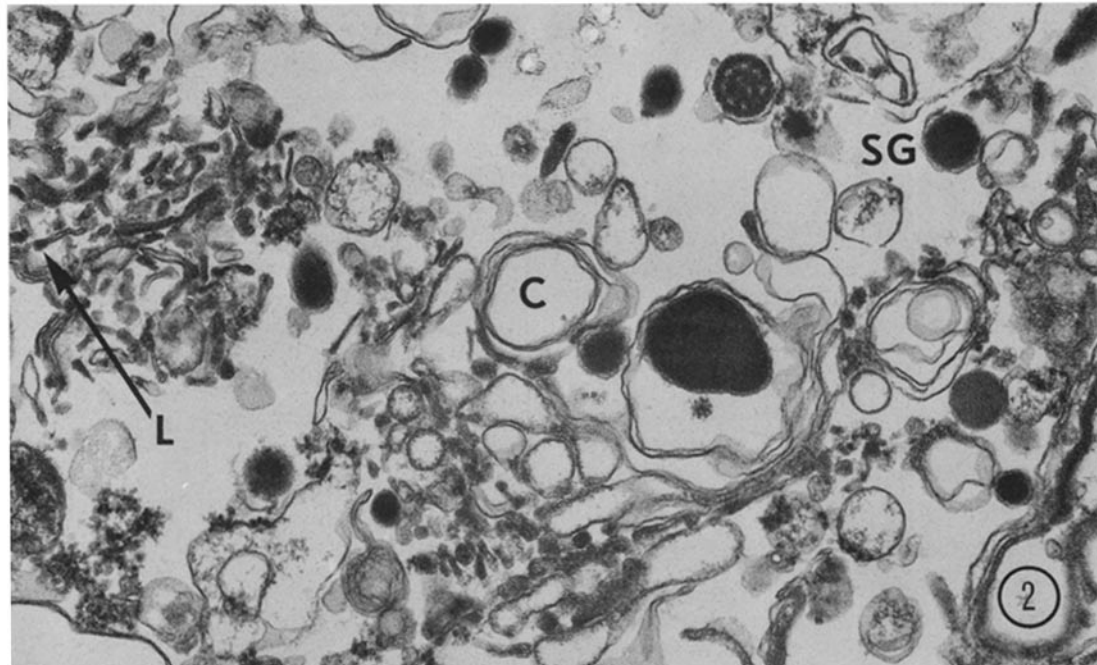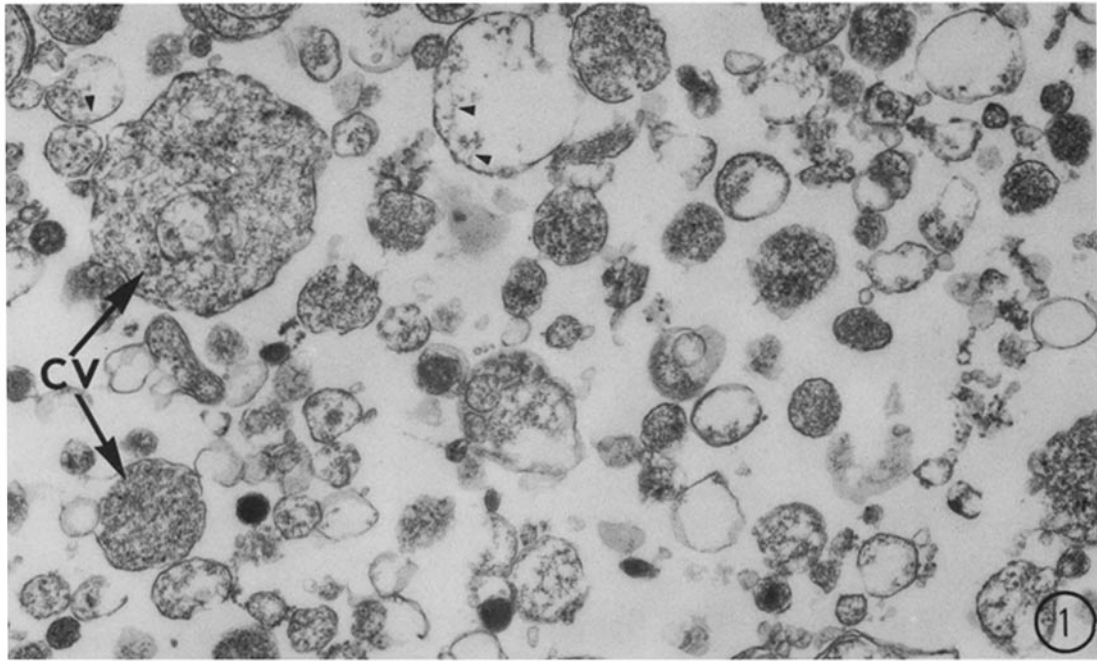The NADH-cytochrome c reductase activity of the microsomal fraction was four times that of the

FIGURE 1   High magnification of the plasma membrane fraction showing the 0.2–1.5-μm closed vesicles with entrapped cytoplasmic remnants (*CV*) and attached microfilaments (arrowheads). × 34,000.

FIGURE 2   High magnification of a section of Golgi fraction processed by the method of Kanaseki and Kadota (10) illustrating flattened lamellae with dense contents and terminal expansions (*L*), dilated cisternae (*C*), and immature secretory granules (*SG*). × 33,000.

TABLE I

Marker Enzymes in Subcellular Fractions of Porcine Adenohypophysis

Fraction	5'-Nucleotidase	Mg++-ATPase	Na+,K+-ATPase	Galactosyl transferase	NADH-cytochrome c reductase	Succinate-cytochrome c reductase
Homogenate	0.26 ± 0.03 (7)	1.4 ± 0.1 (6)	1.8 ± 0.2 (3)	10.2 ± 0.7 (17)	18.1 ± 0.7 (28)	7.9 ± 0.8 (12)
Plasma membranes	4.2 ± 0.5 (6)	4.2 ± 0.5 (6)	6.5 ± 0.6 (5)	15.5 ± 0.3 (10)	30.7 ± 3.1 (11)	1.9 ± 0.3 (10)
Golgi	2.5 ± 0.2 (8)	2.7 ± 0.7 (3)	4.5 ± 0.5 (7)	82.5 ± 6.5 (10)	37.8 ± 5.8 (8)	4.3 ± 0.7 (8)
Mitochondria		2.3 ± 0.1 (3)		30.1 ± 1.6 (3)	5.2 ± 0.1 (3)	36.7 ± 1.8 (3)
Microsomes	1.8 ± 0.1 (6)	3.6 ± 0.2 (3)	4.7 ± 0.2 (3)	23.2 ± 3.0 (5)	71.1 ± 7.4 (6)	1.6 ± 0.2 (6)

The 5'-nucleotidase, Mg-ATPase, and Na,K-ATPase activities are expressed as micromoles P_i per milligram protein per hour. The galactosyl transferase activity is expressed as nanomoles [^{14}C]galactose transferred per milligram protein per hour. The NADH- and succinic-cytochrome c reductase activities are expressed as nanomoles NADH or succinate oxidized per milligram protein per minute. The values represent the mean ± SEM for the number of experiments indicated in parentheses.

homogenate, while the Golgi activity was double, and the plasma membrane activity less than double that of the homogenate. The mitochondrial specific activity actually decreased to a third that of the homogenate. The concentration of RNA (not shown) in the microsomal fraction was five times that of the homogenate, while the plasma membrane and Golgi and mitochondrial fractions had a lesser concentration than the homogenate, consistent with the NADH-cytochrome c reductase enrichments. The succinic-cytochrome c reductase activity was enriched about 4.5-fold in the mitochondrial fraction, while the plasma membrane and Golgi and microsomal fractions had lower specific activities than the homogenate.

Adenylate Cyclase Activity

The basal adenylate cyclase specific activity in the plasma membrane and Golgi fractions was almost six times that of the homogenate (Table II). The enzyme activity was undetectable in the mitochondrial fraction, double the homogenate activity in the microsomal fraction, and unenriched in the nuclear fraction. The purified secretory granule fraction contained one-seventh (48 ± 24 pmol/mg protein/20 min for three experiments) of the homogenate adenylate cyclase activity. NaF at 10 mM caused a tripling of the homogenate and Golgi adenylate cyclase specific activities and a doubling of the plasma membrane fraction activity (Table II). The total recoverable activity in the

plasma membrane fraction was double that of the Golgi fraction since twice as much protein was recovered in the plasma membrane fraction. However, since no attempt was made to optimize the recovery of these fractions, the data may not reflect an accurate picture for the intact cellular system.

The regulation of the adenylate cyclase activity was studied by the in vitro addition of 1 mM $CaCl_2$ or EGTA to the incubation mixture (Table III). The calcium markedly suppressed the enzyme activity in both the Golgi and plasma membrane fractions to the same degree. EGTA slightly stimulated the enzyme activity in both fractions. Both fractions contained cyclic nucleotide phosphodiesterase activity since omission of theophylline from the incubation mixture caused a marked decrease in adenylate cyclase activity.

TABLE II

Adenylate Cyclase Activity in Homogenate, Plasma Membrane, and Golgi Fractions of Porcine Anterior Pituitary

Fraction	Control	+ NaF (10 mM)
Homogenate	320 ± 15 (16)	1,063 ± 64 (9)
Plasma membrane	1,696 ± 205 (10)	3,406 ± 457 (6)
Golgi	1,786 ± 242 (7)	4,868 ± 303 (4)

Adenylate cyclase activity is expressed as picomoles cyclic AMP formed per milligram protein/20 min. The values represent the mean ± SEM for the number of experiments indicated in the parentheses.

TABLE III

The Effect of Calcium and EGTA on the Adenylate Cyclase Activity in Plasma Membrane and Golgi Fractions of Porcine Anterior Pituitary

Fraction	CaCl₂ (1 mM)	Control	EGTA (1 mM)
Plasma membrane	$462 \pm 26 \, (-60\%)$	$1{,}160 \pm 43$	$1{,}577 \pm 128 \, (+36\%)$
Golgi	$327 \pm 17 \, (-56\%)$	741 ± 32	$957 \pm 124 \, (+29\%)$

Adenylate cyclase activity is expressed as picomoles cyclic AMP formed per milligram protein/20 min. The value represents the mean ± SEM of triplicate determinations and the number in the parentheses indicates the percent of inhibition or stimulation of enzyme activity.

DISCUSSION

The present data demonstrate that both the plasma membrane and Golgi subcellular fractions from porcine adenohypophysis were equally enriched in adenylate cyclase activity. To our knowledge, this is the first known account that the Golgi fraction contains this enzyme. It did not seem likely that the enzyme activity in the Golgi fraction was attributable to contamination of that fraction by plasma membranes, or vice versa. Although each fraction was cross-contaminated by the other, each was markedly enriched in either plasma membranes or Golgi membranes as determined by enzyme marker and morphological data. The extensive washing and gradient centrifugations during the purification scheme eliminate simple adsorption of the enzyme to either membrane fraction as an explanation of its presence.

Studies with hypothalamic releasing factors, dibutyryl cyclic AMP, cyclic AMP, theophylline, etc., have implicated the adenylate cyclase system in the stimulation of release of certain hormones from the adenohypophysis (16). Further studies with a variety of procedures known to affect adenylate cyclase activity will be necessary to characterize more fully the separate enzyme activities in adenohypophyseal plasma membrane and Golgi membranes. Since the anterior pituitary is a mixture of different cell types, it is unclear if the enzyme activity exists on the plasma membrane and Golgi membranes of each cell, on the Golgi membranes of one and the plasma membrane of another, or a combination of both. The use of affinity chromatography and electron microscope localization of adenylate cyclase activity may help resolve that issue. The enzyme in both fractions responded identically to the limited number of chemical inhibitors and stimulators thus far applied.

If more exhaustive testing continues to show identical behavior, Golgi adenylate cyclase may prove to be only a precursor to the enzyme in the plasma membrane and serve no other function. If differences in behavior can be demonstrated, then a possible physiological role of Golgi adenylate cyclase in adenohypophyseal function will have to be explored.

SUMMARY

Adenylate cyclase activity was found to be highly enriched (six times homogenate) in the plasma membrane and Golgi subcellular fractions of porcine adenohypophysis but not in the mitochondrial, microsomal, nuclear, or purified secretory granule fractions. The degree of purity of each fraction was determined by both electron microscopy and marker enzyme studies. Neither the Golgi nor the plasma membrane adenylate cyclase activity could be accounted for by contamination with the other fraction, and simple adsorption was ruled out by the extensive purification procedure. The enzyme activity in both fractions was increased by NaF and EGTA and decreased by CaCl₂. The enzyme activity decreased in both fractions if theophylline was omitted from the incubation mixture, and suggested the presence of cyclic nucleotide phosphodiesterase. It is not clear if the adenylate cyclase activity associated with the Golgi fraction is simply a precursor to the plasma membrane enzyme or has its own biological function.

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