PRESENCE OF ADENYLATE CYCLASE ACTIVITY IN GOLGI AND OTHER FRACTIONS FROM RAT LIVER

I. Biochemical Determination

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ABSTRACT

The distribution of adenylate cyclase (AC) in Golgi and other cell fractions from rat liver was studied using the Golgi isolation procedure of Ehrenreich et al. In liver homogenate the AC activity was found to decay with time, but addition of 1 mM EGTA reduced the rate of enzyme loss. The incorporation of 1 mM EGTA into the sucrose medium used in the initial two centrifugal steps of the Golgi isolation method stabilized the enzyme activity throughout the entire procedure and resulted in good enzyme recovery. In such preparations, AC activity was demonstrated to be associated not only with plasma membranes but also with Golgi membranes and smooth microsomal membranes as well. Furthermore, under the conditions used, enzyme activity was also associated with the 105,000 *g* × 90 min supernatant fraction. The specific activity of the liver homogenate was found to be 2.9 pmol · mg protein^{-1} · min^{-1}, the nonsedimentable and microsomal activity was of the same order of magnitude, but the Golgi and plasma membrane activities were much higher. The specific activity of plasma membrane AC was 29 pmol · mg protein^{-1} · min^{-1}. The Golgi activity varied in the three fractions, with the highest activity (14 pmol) in GF_1, lowest activity (1.8) in GF_2, and intermediate activity (5.5) in GF_3. When the Golgi activity was corrected for the presence of content protein, the activity in GF_1 became much higher (9 ×) than that of the plasma membrane while the activities in GF_2 and GF_3 were comparable to that of plasma membrane. In all locations studied, the AC was sensitive to NaF stimulation, especially the enzyme associated with the Golgi membranes. The activities in plasma and microsomal membranes were stimulated by glucagon, whereas the Golgi and nonsedimentable AC were not.

Adenylate cyclase (AC)[1] has been demonstrated to be predominantly associated with plasma membrane fractions prepared from liver and other cells (2, 6–9, 13, 16, 18, 31, 33–35, 37, 40, 53) and is considered to be a marker enzyme for plasma

[1] *Abbreviations used in this paper:* AC, adenylate cyclase; cAMP, 3′5′ cyclic adenosine 3′5′-monophosphate; EGTA, ethylene glycol bis(β-amino ethyl ether)-*N,N′*-tetraacetic acid; ER, endoplasmic reticulum, VLDL, very low density lipoprotein.

membrane. However, the possibility that AC could also be present in other internal cell membranes (endoplasmic reticulum [ER], Golgi) has not been excluded. Literature on this topic has been scanty. In a few cases, the presence of enzyme activity in mitochondria (32, 39), microsomes (21, 32, 39) and nuclei (27, 41) has been described, but the possibility of plasma membrane contamination was not excluded. Therefore, it still remains to be demonstrated whether or not AC is also associated with other membranous structures of the cell. In view of the fact that 5'-nucleotidase, a widely accepted marker enzyme for plasma membrane, has also been shown to be present in Golgi (4, 22) and ER (51, 52) membranes, it is conceivable that AC may also be present in some of the internal cellular membranes.

In the present work,[2] we have taken advantage of the availability of the method established by Ehrenreich et al. (20) to isolate satisfactory Golgi membranes in high yield from rat liver and have looked for and demonstrated the presence of AC in rat liver Golgi membranes. Recovery of the enzyme activity throughout the entire fractionation procedure is also presented.

MATERIALS AND METHODS

Materials

ATP, cAMP, theophylline, and EGTA were obtained from Sigma Chemical Co., St. Louis, Mo., and frozen bovine adrenal cortex from Pel-Freez Bio-Animals, Inc., Rogers, Ark.

Preparation of Rat Liver Golgi and Other Fractions

Golgi and other fractions were obtained from ethanol-treated young (120–150 g) male Sprague-Dawley rats according to the procedure of Ehrenreich et al. (20), which essentially involves three centrifugal steps: a 10,000 g × 10-min spin (spin I) giving pellet I (mostly plasma membrane, mitochondria, and nuclei) and supernate I. The latter was in turn subjected to a 105,000 g × 90 min spin (spin II) resulting in pellet II (microsomal) and supernate II (soluble). Pellet II was rehomogenized and loaded under a discontinuous sucrose gradient. The gradients were spun for 3 h in a swinging-bucket type rotor at 63,500 g (spin III). The three Golgi bands obtained by flotation were referred to in order of increasing density as in the original paper: GF_1 (obtained from the interface between 0.25 M and 0.6 M sucrose), GF_2 (between 0.6 M and 0.86 M), and GF_3 (between 0.86 M and 1.15 M). The material that remained behind after flotation of the Golgi elements was referred to as the residual microsomes. The load zone (1.15 M and 1.17 M sucrose regions) consisted predominantly of smooth microsomes whereas the pellet contained mainly rough microsomes (plus some lysosomes, mitochondria, and peroxisomal cores). A fixed angle rotor (60Ti) was used for the first two spins while a swinging-bucket type rotor (SW 27) was used for the final centrifugation in either a Beckman L5-50 or L5-65 ultracentrifuge (Beckman Instructments, Inc., Spinco Div., Palo Alto, Calif). Unless otherwise stated, the procedure was exactly the same as in the original paper.

Preparation of Rat Liver Plasma Membrane

Plasma membrane was obtained from young male Sprague-Dawley rats (150–200 g) according to the procedure of Stein et al. (43). In the present work, the liver was finely minced in 0.25 M sucrose and passed through a tissue press. Then, it was homogenized in 5 vol of 0.25 M sucrose with 10 strokes of a loose Dounce homogenizer. After filtering through two layers of gauze, it was spun at 1,000 g_{avg} for 10 min. The rest of the procedure was identical to that described in the original paper.

AC Assay

Aliquots of plasma membrane, liver homogenate, and all fractions obtained in the Golgi isolation procedure were incubated for AC activity. Fresh material was used in all cases. The final volume per assay tube was 1 ml. The amount of protein used was, on the average, 1,000 μg for liver homogenate, 500 μg for supernate I, 400 μg for pellet I, 300 μg for supernate II, 500 μg for pellet II, 50 μg for GF_1, 120 μg for GF_2, 320 μg for GF_3, 250 μg for residual microsomes, and 45 μg for plasma membrane. The fraction to be tested was first preincubated at 30°C for 4 min in medium without substrate (consisting of 40 mM Tris maleate buffer, pH 7.4, 2 mM theophylline and 7.5 mM $MgSO_4$) to allow for temperature equilibration as well as equilibration of enzyme with medium. Addition of 2.5 mM ATP started the enzymatic reaction, and the incubation was carried out for 1 min at 30°C. The reaction was stopped by placing the assay tubes in a boiling water bath for 3 min. The tubes were then stored at −20°C overnight for cAMP analysis on the following day. Assays with boiled tissue were used as controls (blanks). The difference between the cAMP level in the experimental (fresh) tube and that in the control (boiled) tube indicated the amount of cAMP formed. When NaF (10 or 20 mM) or glucagon (10 or 20 μg/ml) was used to stimulate AC activity, it was added to the medium before the 4-min preincubation. Under the experimental conditions used, enzyme activity was proportional to time and enzyme concentration.

[2] A preliminary report of these findings was published previously (14).

cAMP Determinations

The saturation assay for cAMP developed by Brown et al. (12) using endogenous binding protein was employed. The binding protein was prepared from frozen bovine adrenal cortex and stored at −20°C. The amount of cAMP formed in each assay tube of AC incubation was measured on duplicate 300-μl aliquots. The amount of cAMP present in each aliquot was calculated from a standard curve constructed each time with 0.1–5 pmol of cAMP. From these data, the amount of cAMP per assay tube could be determined, and the difference between experimental and control tubes indicated the cAMP formed under each experimental condition. Protein determinations were done according to the method of Lowry et al. (28).

RESULTS

AC Activity in Control and Ethanol-Treated Rats

Since the Golgi fractionation procedure of Ehrenreich et al. (20) utilizes alcohol-treated rats (because the yield of Golgi material is much higher than in the normal), it was necessary to establish whether AC activity in such animals differs from that of controls. When AC activity in rat liver fractions was examined in control and ethanol-treated animals, no significant difference was observed in any fraction as shown in Table I. All subsequent determinations were made on fractions from ethanol-treated rats.

AC Activity in Liver Fractions

In the initial experiments, AC activity was tested in rat liver fractions isolated in plain sucrose. Large variations were recorded for each fraction from experiment to experiment, and in a given experiment the activity declined steadily with time. The percent recovery of basal as well as NaF-stimulated AC activity also varied widely from experiment to experiment (data not shown). Storage at −20°C did not prevent the decay of AC activity.

TABLE I

AC Activity in Rat Liver Fractions from Control and Ethanol-Treated Rats in the Presence of 10 mM NaF

	Specific activity		Activity	
	Control	Ethanol-treated	Control	Ethanol-treated
	pmol·mg protein^{-1}·min^{-1}		*(pmol·min^{-1})·g liver^{-1}*	
LH	14	13	3,674	3,107
S_1	11	13	1,334	1,461
P_1	19	20	1,972	2,027
S_2	15	14	863	742
P_2	17	16	603	578

Data represent the average of three experiments with two animals per experimental condition. Assay conditions were as described in Materials and Methods.
Abbreviations used in tables: LH, liver homogenate; P_1, pellet I; P_2, pellet II; S_1, supernate I; S_2, supernate II.

Changes in AC Activity in Liver Homogenate with Time

Since there was some indication of aging, experiments were carried out to determine whether or not enzyme activity was stable over the time interval needed to prepare Golgi fractions. As shown in Fig. 1 (control curve), a decrease in activity with time was observed. By 4 h, no activity could be demonstrated in most cases. As in the case of liver fractions, large variations in the rate of loss of activity were found between experiments.

Effects of Protective Agents on Decline of AC in Liver Homogenates

In view of the decrease observed in AC activity upon aging, attempts were made to reduce the loss by incorporation of protective agents such as ATP,[3] GTP, and EGTA into the homogenization medium.

EGTA: As shown in Fig. 1, when 1 or 2 mM EGTA was added to the homogenate greater survival of enzyme activity was observed than in controls: about one-third of the original activity was still present after 4-h aging. At 3 mM EGTA, AC activity in the homogenate was stimulated by ~100% and loss of activity over 4 h reduced to ~50%. The effects on pellet I were similar (data not shown). Since 1 mM EGTA does not change the AC activity of the liver homogenate, and since further experiments (see below) showed that the activity can be stabilized in subsequent fractions by the rapid separation of pellet I, all further experiments were carried out with 1 mM EGTA.

As shown in Table II, in the presence of 1 mM EGTA the AC activity in the supernate I and pellet II was stabilized and decay of enzyme activ-

[3] ATP was initially introduced since the binding of an enzyme to its substrate is known in many cases to prevent loss of enzyme activity, and in fat cell ghosts ATP is known to provide a stabilizing effect on AC and its response to hormones (36). Therefore, 10 mM ATP was added to the homogenate; however, the presence of ATP was found to interfere with the AC assay.

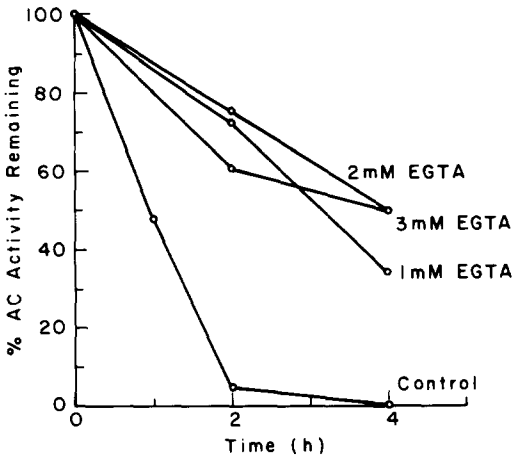

FIGURE 1 This figure shows the aging of basal adenylate cyclase activity in rat liver homogenate. Data represent average of two experiments using one animal per experimental condition. Liver homogenate was kept on ice and stored in the cold room for the entire length of the experiment. An aliquot was removed at each time interval for AC incubation and cAMP analysis. Assay conditions were as described in Materials and Methods.

ity with time was no longer observed. As in the case of liver homogenate, the AC activity in pellet I still decreased with time. However, the rate of loss of enzyme activity was slower than that observed in liver homogenate under similar conditions.

Effects of EGTA on Decline in AC Activity in Liver Fractions

On the basis of the protective effect of 1 mM EGTA on AC in liver homogenate and in the supernate and pellet of the 10,000 *g* × 10 min spin as described above, this agent was introduced into the Golgi fractionation procedure. Initially, 1 mM EGTA was added to the sucrose medium throughout the entire Golgi isolation procedure. However, in the final spin the Golgi material was observed to aggregate into sheets visible to the naked eye. As a result, most of the material was located in the band referred to as "GF_3," whereas much less material was recovered in the "GF_2" band and hardly any in "GF_1." Subsequently, 1 mM EGTA was incorporated into the sucrose medium of the initial two spins but removed from the final spin by rehomogenizing pellet II in EGTA-free sucrose and using a EGTA-free sucrose gradient. Under these circumstances, the usual distribution of Golgi material in the gradient was maintained. Furthermore, as shown in Table III, the AC activity demonstrated in all fractions of the Golgi isolation procedure was very stable. This was true of both basal and NaF-stimulated activity. Additionally, there was almost complete recovery of enzyme activity.

Distribution of AC in Liver Fractions

Once stabilization of enzyme activity over the period required for Golgi isolation had been achieved with EGTA, a series of seven experiments was carried out in which AC activity was measured in rat liver fractions in the presence of this protective agent. The results of these experiments are given in Table III. All the fractions including the 105,000 *g* supernate (supernate II) contained measurable AC activity, but the only fractions in which the specific activity was consistently and substantially enriched (especially in the presence of NaF) over the original homogenate were the Golgi fractions. The distribution (%) of AC activity in rat liver cell fractions is also shown in Table IV. About two-thirds of the total activity sediments with pellet I (which contains most of the plasma membrane as well as nuclei and mitochondria) whereas, suprisingly, one-third of the activity is located elsewhere. Approximately 16–18% of the basal and NaF-stimulated activity is associated with supernate II, while the remainder of the activity (12% of the basal and 19% of the NaF-stimulated activity) sediments with pellet II (the microsomes). Of the latter, about 1.4% of the total basal and 2.3% of the NaF-stimulated activity is associated with the Golgi fractions while

TABLE II

Aging of Basal AC in Rat Liver Fractions in the Presence of 1 mM EGTA

	Specific activity			Activity		
	P_1	S_1	P_2	P_1	S_1	P_2
	pmol·mg protein^{-1}·min^{-1}			*(pmol·min^{-1})·g liver^{-1}*		
0 h	5.3	1.6	2.1	699.4	172.5	67.4
2 h	4.2	1.4	2.1	584.2	150.0	67.4
4 h	3.3	1.4	2.1	437.5	150.0	67.4
6 h	2.8	1.6	–	374.7	172.5	–

Data represent average from two experiments with three animals per experiment. Cell fractions were kept on ice and stored in the cold room during the entire span of the experiment. An aliquot was removed at each time interval for AC incubation and cAMP analysis subsequently. Assay conditions were as described in Materials and Methods.

TABLE III

AC Activity in Rat Liver Cell Fractions in the Presence of 1 mM EGTA

	Specific activity		Activity		Percent recovery	
	Protein	Basal	10 mM NaF	Basal	10 mM NaF	Basal	10 mM NaF
	mg protein · g liver^{-1}	*pmol · mg protein*$^{-1}$ · *min*$^{-1}$		*(pmol · min*$^{-1}$*) · g liver*$^{-1}$			
LH	248.2	2.9	19.8	699.0	4891.4		
S_1	108.5	2.1	13.2	214.0	1342.9	95*	97.5*
P_1	117.4	3.6	27.4	448.0	3425.6		
S_2	56.8	2.5	17.0	128.0	798.1	99.1‡	128.5‡
P_2	40.6	2.4	26.4	84.0	926.9		
Residual microsomes	30.0	2.9	34.0	70.0	844.6		
GF_1	0.1	14.0	131.6	2.0	18.7	94.9§	103.3§
GF_2	0.3	1.8	50.9	0.7	19.0		
GF_3	1.1	5.5	61.8	7.0	75.0		

Golgi fractionation was carried out with 1 mM EGTA incorporated into the sucrose medium employed in the first two spins only. Before the final spin, pellet II was rehomogenized in EGTA-free sucrose which was also used to prepare the discontinuous gradient for the final spin. Data represent average of seven experiments with four-six animals per experiment. An aliquot was removed from each subfraction for AC incubation and subsequent cAMP determination.

* Calculated from values of activity · g liver^{-1} ($[S_1 + P_1]/LH \times 100$)%.

‡ Calculated from values of activity · g liver^{-1} ($[S_2 + P_2]/S_1 \times 100$)%.

§ Calculated from values of activity · g liver^{-1} ($[RM + GF_1 + GF_2 + GF_3]/P_2 \times 100$)%.

TABLE IV

*Distribution of AC Activity in Rat Liver Fractions in the Presence of 1 mM EGTA**

		AC activity	
	Protein	Basal‡	10 mM NaF§
	%	%	%
LH	100.0	100.0	100.0
S_1	43.7	30.6	27.5
P_1	47.3	64.1	70.0
S_2	22.9	18.3	16.3
P_2	16.4	12.0	18.9
Residual microsomes	10.4	10.0	17.3
GF_1	0.04	0.3	0.4
GF_2	0.12	0.1	0.4
GF_3	0.44	1.0	1.5

* Calculated from values for activity/g liver given in Table III.

‡ Recovery of basal AC activity was 94%.

§ Recovery of NaF-stimulated activity was 106%.

10% of the total basal and 17% of the NaF-stimulated activity is present in residual microsomes.

AC Activity in Golgi Fractions

The specific activity of both basal and NaF-stimulated AC varies among the Golgi fractions and is highest in GF_1 and lowest in GF_2 which has a specific activity less than that of the homogenate (See Table III). Parallel cytochemical localization of AC (15) indicated the activity to be associated with Golgi membranes as expected since AC is usually considered to be a membrane enzyme. Therefore, the specific activity obtained from Golgi fractions should be corrected for the presence of content protein to obtain a value which can be compared to that of plasma membrane. An attempt was made to release the contents of Golgi elements with the French pressure cell in order to measure the AC activity of Golgi membranes directly. However, preliminary experiments resulted in a loss of enzyme activity. Thus, we utilized the data of Ehrenreich et al. (20) and the improved but unpublished data of Howell and Palade, indicating that ~95% protein of a mixed fraction of GF_1 and GF_2, and ~75% of GF_3 represent content protein and the remainder (5% and 25%, respectively) membrane protein. When corrected for content protein, the activity of all three Golgi fractions is seen to be highly enriched over that of the homogenate, resulting in an increasing activity gradient from GF_3 to GF_1 (Table V). The basal activity in GF_3 is about seven times that of the homogenate, in GF_2 12 times, and in GF_1 90 times that of the homogenate. The same is true of the NaF-stimulated activity.

In Table V the specific activity of AC in Golgi membranes is compared to that of plasma mem-

brane. The data indicate that the specific activity of both basal and NaF-stimulated AC of GF_1 is much greater than that of plasma membrane whereas that of GF_2 and GF_3 is comparable to that of plasma membrane.

Effect of Glucagon on Golgi AC

As shown in Table V the Golgi activity differs from plasma membrane activity in that there is no measurable stimulation in the presence of glucagon (10 μg/cc). In order to investigate further the lack of response to glucagon of Golgi AC, the effect of varying the concentration of glucagon was studied. As shown in Table VI, an amount of glucagon sufficient to maximally stimulate AC in liver homogenate, microsomal (pellet II) and residual microsomal fractions did not stimulate Golgi AC. Similarly, the same concentrations of glucagon failed to stimulate AC in supernate II (data not shown).

Effect of Varying Concentrations of NaF on Golgi AC

While AC in every liver fraction tested responds to NaF stimulation, the activity in most cases (homogenate, supernate II, pellet II and residual microsomes) reaches a plateau level around 6 mM NaF (data not shown). The Golgi AC response to NaF is shown in Fig. 2. In the case of Golgi activity, there is variation among the subfractions. GF_1 shows the greatest response to NaF and GF_3 the least, with GF_3 reaching a plateau around 6 mM NaF and GF_1 not until 16 mM NaF is reached.

AC Activity in Microsomal Fractions

The AC activity present in the pellet (predominantly rough microsomes) and the load zone (predominantly smooth microsomes) of the final Golgi centrifugation (spin III) was tested. Activity was shown to be associated with aliquots from the load zone, i.e., the smooth microsomes, only (Table VII). No activity could be demonstrated within the rough microsomes.

Nonsedimentable AC

Supernate II was subjected to further centrifugation at 131,000 g_{max} for 22 h to determine whether or not it was sedimentable under these

TABLE V

Specific Activities of Golgi and Plasma Membrane AC

	Basal		10 mM NaF		10 μg/cc glucagon	
	Membrane and content	Membrane	Membrane and content	Membrane	Membrane and content	Membrane
	pmol·mg protein^{-1}·min^{-1}		*pmol·mg protein^{-1}·min^{-1}*		*pmol·mg protein^{-1}·min^{-1}*	
Plasma membrane	–	29 (30*)	–	191 (192*)	–	203 (333*)
GF_1	14.0	280‡	132	2,640‡	14	0‖
GF_2	1.8	36‡	51	1,020‡	0.6	0‖
GF_3	5.5	22§	62	248§	5.5	0‖

* Value of Pohl et al. (31).

‡ GF_1 and GF_2: based on content of ~95% (Howell and Palade, personal communication).

§ GF_3: based on content protein of ~75%. (Howell and Palade, personal communication).

‖ Complete loss of AC activity after treatment with the French Press.

TABLE VI

Specific Activity of AC in Rat Liver Fractions in the Presence of Varying Concentrations of Glucagon

Glucagon	0 μg/cc	0.01 μg/cc	0.1 μg/cc	1 μg/cc	10 μg/cc
	pmol·mg protein^{-1}·min^{-1}			*pmol·mg protein^{-1}·min^{-1}*	
LH	3.3	10.0	9.2	11.3	12.2
P_2	3.8	3.8	10.7	18.9	15.2
Residual microsomes	2.8	4.2	8.4	11.2	9.8
GF_1	15.9	15.9	15.9	15.9	15.9
GF_2	2.0	2.0	2.0	0.7	0.7
GF_3	6.3	5.3	6.3	7.4	6.3

Data represent average of two experiments with three–four animals per experiment. Assay conditions were as described in Materials and Methods.

conditions. After such a spin, AC activity remained in the supernate.

DISCUSSION

The unstable nature of the AC system is widely recognized among investigators in this field. Rodbell and Krishna (36) reported that the AC response to hormone is unstable in the fat cell ghost, decaying at a rate of about 50% in 4 h even when ghosts are maintained at 0°C. We were not able to preserve enzyme activity even when fractions were stored at −20°C. Thus, it is not surprising to find that the activity in liver homogenate decays with time. The fact that 1 mM EGTA is useful in preserving the enzyme led one to suspect that Ca^{++} may be involved in the process. In fact, the enzyme activity begins to stabilize after the removal of pellet I which contains most of the mitochondria and thus probably also most of the Ca^{++}. The effect of Ca^{++} on AC varies according to the tissue. Under most conditions, AC is inhibited by Ca^{++} (30, 44). Inhibition of AC by Ca^{++} has been demonstrated in toad bladder (23), fat cell ghosts (2, 19, 36, 50), adrenal mitochondria (3), a detergent-dispersed AC from rat brain (24), pars distalis of dogfish pituitary (17), and turkey erythrocyte ghosts (42). Even in cases where Ca^{++} is essential for stimulation of AC by hormones, a high concentration of Ca^{++} is also inhibitory. This has been demonstrated in the MSH-induced activation of AC in *Xenopus laevis* (49), the ACTH-activated adrenal AC (25), as well as the stimulation of AC by oxytocin in frog bladder epithelial cells (10).

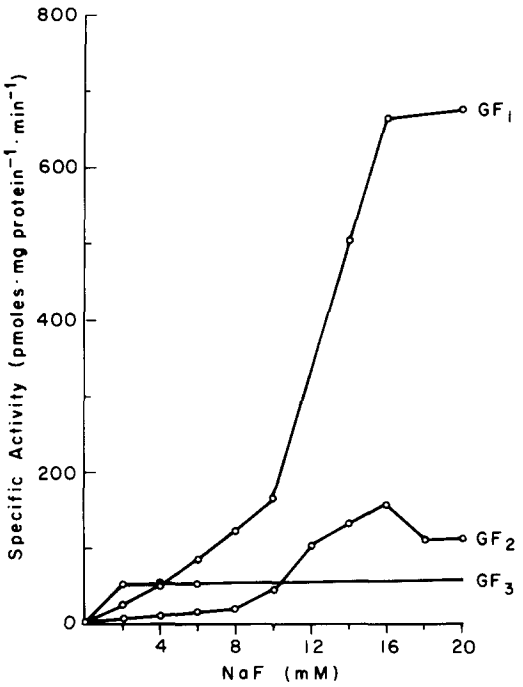

FIGURE 2 This figure shows the variations in the response of the three Golgi fractions to NaF. GF_1 shows the greatest response and GF_3 the least.

The present work suggests that Ca^{++} is also inhibitory to rat liver AC. Presumably, 1 mM EGTA removes enough Ca^{++} from the cell fractions so that the enzyme activity is stabilized during the entire Golgi isolation procedure. However, other explanations for the protective effect of EGTA cannot be excluded. These include the possibility that pellet I may contribute inhibitors, the release of which is blocked by EGTA; inhibition of Ca^{++}-dependent proteolytic activity; or removal of ions (iron or copper) which promote oxidation of sulfhydryl groups essential for activity

TABLE VII

AC Activity in Microsomes

	Basal		10 mM NaF	
	Specific activity	Activity	Specific activity	Activity
	pmol · mg protein⁻¹ · min⁻¹	*(pmol · min⁻¹) · g liver⁻¹*	*pmol · mg protein⁻¹ · min⁻¹*	*(pmol · min⁻¹) · g liver⁻¹*
Smooth microsomes	2.7 (2.5–2.9)	55.9 (54.3–57.4)	14.6 (13.5–15.7)	304.5 (293.1–315.9)
Rough microsomes	0	0	0	0

Smooth microsomes were obtained from 1.17–1.15 M region of the gradient and rough microsomes from the pellet of the final spin. Assay conditions were as described in Materials and Methods. Data represent average of two experiments with six animals per experiment, and numbers in parenthesis give the range.

of the enzyme. Whatever the explanation, the fact is that by using EGTA it is possible to obtain a meaningful distribution of AC activity in rat liver cell fractions (Table III).

Golgi AC

The presence of AC activity in the Golgi apparatus has been briefly communicated by ourselves (14) in rat liver and by McKeel and Jarett (29) in porcine adenohypophysis. The present paper together with the one that follows (15) represents a complete version of the previous presentation. While the AC in the other fractions obtained from the Golgi isolation procedure has a specific activity similar to that of liver homogenate, the Golgi fractions alone demonstrated enrichment of AC activity (Table III). This activity is very sensitive to fluoride stimulation but does not respond to glucagon even when tested over a wide range of hormone concentrations (Table VI). The lack of response to glucagon may be due to the absence of glucagon receptors or, more likely, glucagon fails to traverse the membrane to reach receptors which, like insulin receptors (5), would be expected to be associated with the inside of Golgi membranes. The inside of the Golgi membrane, upon secretion of very low density lipoprotein (VLDL)-filled droplets, becomes the outside of the plasma membrane (see reference 20). Preliminary attempts to break open Golgi elements by treatment with the French press in order to expose glucagon receptors led to loss of enzyme activity. Since all other fractions including liver plasma membranes respond to glucagon stimulation (Table VI), the lack of glucagon response in the Golgi fractions indicates that the enzyme activity is not due to plasma membrane contaminants. Furthermore, cytochemical localization of AC confirmed the absence of plasma membrane contaminants and demonstrated the association of enzyme activity with morphologically identifiable Golgi membranes (15).

Since AC is associated with Golgi membranes, the extremely high content protein of Golgi elements must be taken into consideration when determining the specific activity of the enzyme. When corrected for content protein, the specific activity of the Golgi fractions became very high indeed (Table V). GF_1 has an activity of about nine times that of plasma membrane while the activities of GF_2 and GF_3 are comparable to the plasma membrane value. The data, therefore, establish a compositional overlap between Golgi and plasma membranes. Since the fate of at least some Golgi membranes is to be inserted into the plasma membrane at the time of secretion of the content of the VLDL-filled droplets, it is possible that the Golgi AC is a precursor of the plasma membrane AC. In fact, the pattern of response to increasing doses of NaF stimulation of AC in GF_1 (Fig. 2) is very similar to that of liver plasma membrane AC reported by Pohl et al. (31). However, kinetic data are needed to establish or refute this type of relationship.

Nonsedimentable AC

The present investigation clearly indicated the existence of a nonsedimentable form of AC in the 105,000 *g* supernate (S_2). However, it is not certain whether AC is present in the cytoplasmic matrix under physiological conditions or whether it is an artifact of the homogenization procedure and represents activity extracted from plasma membrane or other membranes. In any case, when this fraction is subjected to further centrifugation at 131,000 g_{max} for 22 h, AC activity can still be demonstrated in the supernate, indicating that it is indeed nonsedimentable. A soluble or nonsedimentable AC has been reported previously in Yoshida ascites hepatoma (48) and in rat testis (11) as well as in *Escherichia coli* (47) but has not been reported in normal liver. In the case of the hepatoma, most of the AC activity exists in the soluble form. Solubilization of AC from homogenates of heart (26, 45), brain (24, 45), liver (45), and liver membranes (38, 46) has been achieved using nonionic detergents. It would be of interest to compare the properties of the nondetergent extracted, nonsedimentable AC we have isolated from liver to those of AC extracted from liver membranes with detergents. Incidentally, it could be argued that the Golgi AC activity is due to the adsorption of the nonsedimentable enzyme onto Golgi membranes. However, the highest specific activity of the Golgi fractions vs. the low activity of supernate II as well as the difference between their fluoride responses points to the contrary.

Microsomal AC

Enzyme activity at the same level as that in liver homogenate was also demonstrated in the residual microsomal fraction (Table III). This was further demonstrated to be associated only with the

smooth microsomes (Table VII) since no enzyme activity could be detected in the rough microsomes. The absence of AC activity in the rough microsomes may be real or due to loss of enzyme activity as a result of unknown factors. For example, the enzyme activity could be destroyed upon rehomogenization of the pellet by release of lysosomal enzymes into the medium since lysosomes were frequently observed in this fraction. Thus, it will be of interest in the future to determine AC activity in purified rough microsomes (cf. reference 1). The activity associated with smooth microsomes could be indigenous to these elements or could be due to contamination by plasma membrane fragments. However, our cytochemical results reported in the following paper (15) demonstrate that the activity is indigenous to microsomes.

CONCLUSION

The present work has demonstrated that AC is more widely distributed in liver cell fractions than previously assumed. AC is present at high specific activity in Golgi membranes and at lower activity in smooth microsomes and in the 105,000 g_{max} supernate. Therefore, contrary to common belief, AC cannot be considered as a specific plasma membrane marker enzyme, at least in the case of rat liver.

The constructive criticism of Dr. G. E. Palade throughout this work is appreciated. We are grateful to Doctors J. Kebabian and Y. Clement-Cormier for their assistance in setting up the cAMP assay.

This work was supported by research grant (AM 17780) from the National Institutes of Health, U. S. Public Health Service.

Received for publication 1 December 1975, and in revised form 9 April 1976.

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