Vol. 108, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 30, 1982 Pages 567-575

SOLUBILIZATION OF HORMONE-RESPONSIVE ADENYLATE CYCLASE FROM HUMAN RENAL CORTEX*

Frederick G. Hamel and Sherry F. Queener ‡

Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana 46223

Received August 5, 1982

Adenylate cyclase activity from human renal cortical plasma membranes remained in the 100,000 xg supernatant (2 hrs) following treatment with 0.25% Lubrol PX in 10mM Tris buffer (pH 7.45), 1 mM EDTA, 0.25 M sucrose, and 5 mM NaF. Solubilization decreased total adenylate cyclase activity by at least one-half; responsiveness to calcitonin, glucagon and guanyl nucleotides, but not to parathyroid hormone, was preserved. Glucagon and calcitonin-stimulated adenylate cyclase eluted near the void volume on Sephadex G200 columns; two other peaks of non-hormone stimulated activity eluted later.

Through a collaborative arrangement with the National Diabetes Research Interchange (3624 Science Center, Philadelphia, PA 19104) we have obtained human renal cortex for the purpose of characterizing the adenylate cyclase activity in this tissue. We herein report the solubilization of adenylate cyclase activity responsive to glucagon, calcitonin, and 5'-guanylylimidodiphosphate (Gpp[NH]p) from this tissue. Human tissues have not previously been used for extensive characterization of solubilized adenylate cyclase. Moreover, the properties of this enzyme contrast to all other systems reported.

Solubilized adenylate cyclase in which the hormone responsiveness could be restored by removal of the detergent and/or by

*This work was carried out under a Grant-in-Aid from the American Heart Association, Indiana Affiliate, Inc., and PHS grant R01 AM 21289.

‡ To whom correspondence and reprint requests should be addressed

0006-291X/82/180567-09$01.00/0

the addition of lipids has been reported for a few tissues (1-8). One report suggests that glucagon- and epinephrine-stimulated adenylate cyclase can be obtained directly in solubilized form (9) rat liver plasma membranes treated with Triton-305. However, other workers using Lubrol PX instead of Triton-305 in rat liver plasma membranes obtained active glucagon receptor and guanyl nucleotide-stimulated adenylate cyclase, but the adenylate cyclase did not respond to glucagon (5,6). Moreover, the solubilization with Triton-305 could not be repeated with subsequent lots of detergent (Daniel R. Storm, personal communication). Previous studies from our laboratory have described an adenylate cyclase solubilized from porcine renal cortical plasma membranes by Lubrol PX treatment in the presence of NaF; unlike the enzyme described in this paper, solubilized porcine adenylate cyclase lost responsiveness to glucagon and parathyroid hormone (10). In both the porcine and human systems the hormone response that survived solubilization with Lubrol also showed enhancement by low levels of NaF.

METHODS AND MATERIALS

Porcine calcitonin was the generous gift of Dr. R. Schleuter, Armour Pharmaceutical Co. Bovine parathyroid hormone (1425 MRC units/mg, Lot 1508G-0U7) was purchased from Inolex Laboratories. Crystalline glucagon was obtained from Eli Lilly and Co. GTP and (Gpp[NH]p) were from BoeringerMannheim. [Alpha-^{32}P]ATP (30 to 50 Ci/mmol) and cyclic [8-^{3}H]AMP (5 to 15 Ci/mmol) were purchased from New England Nuclear Corp and Amersham.

Adenylate cyclase assay--Adenylate cyclase (ATP pyrophosphate-lypase [cyclizing], EC 4.6.1.1) activity was assayed by a modification of the technique of Salomon (11) in the reaction mixture previously used in our laboratory (10,12), except that in the current studies 0.96 mM ATP was used as the final concentration of substrate in the assay since this concentration of ATP gave maximal activity of the adenylate cyclase from the human membranes. The assays contained 75 to 150 µg of protein/100 µl of reaction mixture and were incubated for 15 min at 37°C. Under these conditions with these membranes, cyclic AMP formation was linear with respect to time for at least 20 min and with respect to protein up to 200 µg. Linearity of cyclic AMP formation with respect to time was observed not only for basal conditions but also for NaF and hormone-stimulated conditions. Protein was measured by the biuret reaction with bovine serum albumin (crystallized, lyophilized from Sigma) as a standard (13).

Acquisition of human samples--Tissue donors were caucasian males with no history of chronic disease. The kidneys were harvested according to standard protocols used for kidney transplantation and flushed with Sacks·II or Collins II solutions. The kidneys donated to the National Diabetes Research Interchange and used in these studies were judged not suitable for transplantation by the attending surgeon on the basis of length of ischemia, quality of perfusion, or unfavorable structure. Perfused kidneys were dissected with a scalpel to yield sections of renal cortex and quickly transferred to chilled preservation buffer. These sections were further dissected to yield tissue cubes of ca. 1 cm^3. The tissue was transferred to Eagle's minimal essential medium with 100 µg/ml gentamicin, frozen and shipped on dry ice from Philadelphia by air. Transit times have generally been less than 8 hours and the tissue remains frozen during shipment.

Membrane preparation--Partially purified plasma membranes were prepared by the procedure of Marx et al. (14). Comparison of purifications from frozen tissue (-70°C) and fresh renal cortex showed the frozen tissue to be equivalent or slightly superior to fresh tissue in terms of yield of activity. Mean yields of basal and fluoride-stimulated adenylate cyclase were 5845 and 74,109 units/g wet weight, respectively, for 6 samples shipped at -70°C versus 2368 and 42,117 units/g wet weight for 3 samples shipped at 4°C. No difference in hormone responsiveness between membranes derived from fresh or frozen tissue could be detected. Patterns of hormone responses were also consistent from sample to sample, suggesting small variability in the normal population from which our tissues were derived. For all the studies on solubilized adenylate cyclase, plasma membranes derived from tissue stored at -70°C were used.

RESULTS

Low concentrations of the non-ionic detergent Lubrol PX in the presence of NaF release adenylate cyclase activity from plasma membranes prepared from human renal cortex (Table I). The enzyme activity that is released retains the ability to be stimulated by the peptide hormones calcitonin and glucagon, as well as the guanyl nucleotide analog, Gpp[NH]p. Responsiveness to parathyroid hormone is lost following detergent treatment. Very little adenylate cyclase activity could be detected in the pelleted membranous material remaining after detergent treatment, although significant amounts of protein were present in that fraction. Centrifugation for 30 min at 6500 xg or for 2 hrs at 100,000 xg yielded supernatant fluids with similar adenylate cyclase content. Simple dilution of the plasma membrane preparations in the presence of NaF leaves the bulk of adenylate

Table I

Recovery of hormone-responsive adenylate cyclase activity in supernatant fluid following treatment of plasma membranes with Lubrol PX in the presence of NaF.

	Solubilization procedure for partially purified plasma membranes (fr 6)					
	1: Incubation 30 min in Buffer A[a]; no centrifugation	2: Suspended in Buffer A[a], 5 mM NaF, 0.25% Lubrol PX; 30 min at 6500 xg			3: Suspended in Buffer A[a], 5 mM NaF, 0.25% Lubrol PX; 2 hr at 100,000 xg	
Adenylate cyclase assay condition	Original suspension	Original Suspension[c]	Pellet[c]	Supernatant[c]	Pellet[c]	Supernatant[c]
	Adenylate cyclase activity, pmol cAMP formed/15 minutes/mg protein					
Basal	57 ± 3	86 ± 3	16 ± 73	92 ± 13	<5	155 ± 26
Parathyroid hormone 5 μ M	1446 ± 13[b]	84 ± 17	<5	101 ± 9	---	---
Pork calcitonin 10 units/ml	493 ± 2[b]	220 ± 11[b]	92 ± 46	238 ± 8[b]	<5	349 ± 18[b]
Glucagon 1 unit/ml	987 ± 5[b]	478 ± 24[b]	<5	499 ± 23[b]	16 ± 19	487 ± 25[b]
Gpp[NH]p 10mM	1020 ± 15[b]	318 ± 11[b]	<5	399 ± 19[b]	<5	351 ± 14[b]
NaF 10mM	1280 ± 20[b]	398 ± 26[b]	159 ± 21	389 ± 20[b]	12 ± 32	424 ± 36[b]
Protein recovery	100%	100%	14%	86%	43%	57%

[a]Buffer A = 10mM Tris-HCl (pH 7.45), 1 mM EDTA, 0.25 M sucrose.
[b]Statistically significant differences from basal activity within each column (Student's test).
[c]1.25 mM NaF is carried over into the adenylate cyclase assay for all samples solubilized in the presence of NaF.

Partially purified plasma membranes (fr 6) were divided into three aliquots. The first aliquot was diluted in Buffer A to 5mg/ml membrane protein, held on ice 30 min, then assayed (first column). The second aliquot was diluted in Buffer A and adjusted to a final concentration of 5 mg/ml membrane protein, 5mM NaF, and 0.25% Lubrol PX; an aliquot of the suspension was centrifuged 30 min at 6500 xg (4°C) and the remainder was assayed as the suspension (columns 2-4). The third aliquot was diluted as the second aliquot but the entire suspension was centrifuged 2 hrs at 100,000 xg (4°C); pellet and supernatant were assayed (columns 5 & 6).

Table II

Effect of NaF ± Lubrol PX on solubilizing adenylate cyclase.

Sample	Protein	Yield	Adenylate cyclase total units (pmol cAMP/15') Basal	PCT 10u/ml	NaF 10mM
	mg				
Partially purified plasma membranes (fr 6)	5.0	---	294	449	3830
fr 6 in Buffer A with 5mM NaF[a]	4.8	100%	1674	4583	3756
Pellet[a]	3.7	77%	1705	3922	3256
Supernatant[a]	1.3	24%	81	286	234
fr 6 in Buffer A with 5mM NaF, 0.5% Lubrol PX[a]	5.0	100%	238	816	972
Pellet[a]	1.2	24%	<5	<5	<5
Supernatant[a]	4.3	76%	258	640	809

[a]All of these samples have a carryover of 1.25mM NaF into the adenylate cyclase assay.

Partially purified plasma membranes (fr 6) from sample 006-01 were divided into three aliquots. The first aliquot was diluted in Buffer A (Tris 10mM pH 7.45, EDTA 1mM, sucrose 0.25M) to 5mg/ml membrane protein, held on ice, then assayed as shown (data in row 1). The second aliquot was diluted in Buffer A and NaF to yield 5mg/ml membrane protein, and 5mM NaF. The aliquot was divided in half and one half was assayed directly (row 2); the other half was centrifuged at 6500xg for 30 min. Pellet and supernate were separated and the pellet resuspended in Buffer A, 5mM NaF before assay (rows 3 and 4). The third aliquot was treated as the second except 0.5% Lubrol PX was included in the membrane dilution.

cyclase activity in the pelleted membranes, following centrifugation at 6500 xg for 30 minutes, whereas dilution in the presence of both NaF and 0.5% Lubrol PX both releases adenylate cyclase activity and lowers the recovery of total adenylate cyclase units (Table II). There is also an apparent increase in adenylate cyclase response to calcitonin when the partially purified plasma membranes are assayed in the presence of submaximal stimulating levels of NaF.

The ability of 1.25mM NaF to potentiate the response of adenylate cyclase to other hormones was tested in partially purified plasma membranes (Table III). In this experiment, NaF greatly enhanced the response of the enzyme to glucagon and calcitonin but had no effect on parathyroid hormone response.

Table III

Effect of low NaF concentrations on hormone responses of human renal adenylate cyclase.

Assay Condition	Adenylate Cyclase Activity: Partially Purified Membranes	Partially Purified Membranes + 1.25mM NaF
	pmoles cAMP formed /15'/mg protein	
Basal	165±16	576±33
NaF 10mM	2276±58 (13.8)	2122±33 (3.68)
PTH 5μM	2339±39 (14.2)	2444±35 (4.2)
Calcitonin 4u/ml	191±12 (1.16)	2914±66 (5.1)
Glucagon 0.1u/ml	338±13 (2.05)	3015±62 (5.2)

Partially purified plasma membranes were divided into 2 aliquots. One was diluted to 4mg/ml protein in Buffer A (Tris 10mM pH 7.45, 1mM EDTA, 0.25M sucrose); the second was diluted identically except the final dilution also contained 5mM NaF. The samples were then assayed for adenylate cyclase activity by standard procedures. The numbers in parentheses are the ratios between stimulated and basal activities.

Adenylate cyclase activity solubilized as described in Table I could be resolved into several peaks of activity by Sephadex G200 chromatography (Fig. 1). Catalytic subunit activity (as measured by forscolin-stimulated activity) is observed in 3 distinct peaks, one near the void volume. Fluoride-stimulated activity is found in the large molecular weight peak and in the region between the smaller two peaks of forscolin-stimulated activity. Calcitonin and glucagon responsive adenylate cyclase activity, not measured on the column shown, was associated primarily with the peak nearest the void volume in the three other columns run under these conditions.

DISCUSSION

Sodium fluoride enhances the response of the human renal cortical adenylate cyclase to calcitonin and glucagon (Table II), but not to parathyroid hormone. This enhancement of response occurs at NaF concentrations below those required to maximally

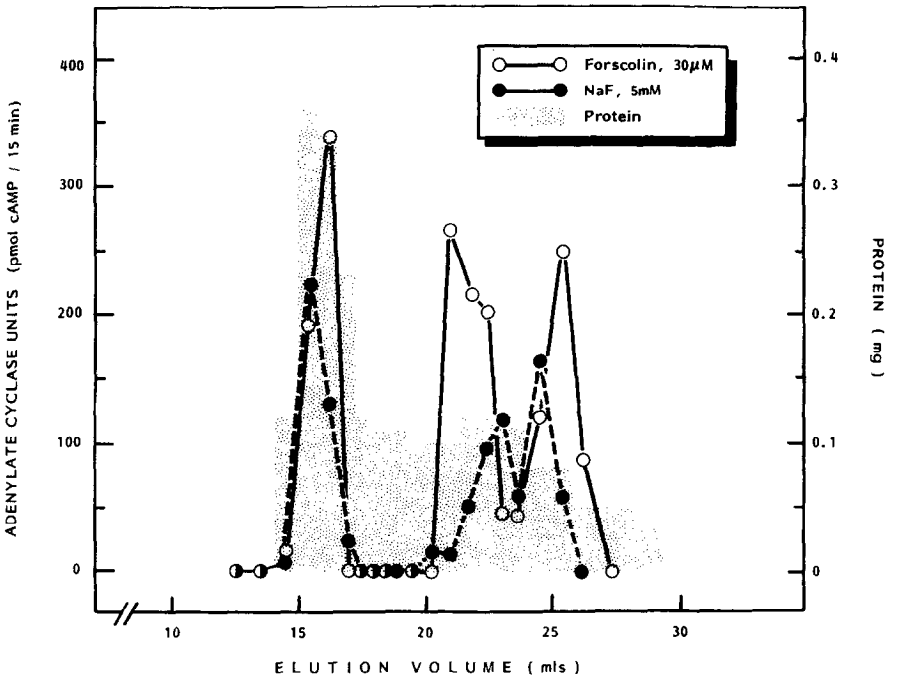

Fig. 1 Sephadex G200 column chromatography of solubilized human renal cortical adenylate cyclase. Sephadex G200 (particle size 40-120 μ) column (1 x 58 cm) was loaded with 0.9ml of a 37,00 xg supernatant fluid obtained from partially purified plasma membranes (code 092-55.ON) by treatment with 0.25% Lubrol PX and 5mM NaF in Buffer A (10mM Tris pH 7.45, 1mM EDTA, 0.25M Sucrose). Elution was with Buffer A containing 5mM NaF and 1mM dithiothreitol. The void volume of the column as determined by Dextran BLue elution was 14 mls.

stimulate the enzyme. The synergistic interaction between NaF and calcitonin has also been observed in porcine adenylate cyclase (12) but no similar enhancement of glucagon or parathyroid hormone responsiveness was observed with the enzyme from that tissue. Interestingly, the adenylate cyclase forms in both tissues that show enhancement of hormonal activation by NaF are also the forms that survive solubilization by Lubrol PX. These observations suggest that the porcine and the human calcitonin-responsive adenylate cyclase and the human glucagon-responsive adenylate cyclase from kidney represent a class of adenylate cyclases that differs from other classes in the degree of interaction with the plasma membrane in which they are embedded and/or

in the degree of interaction between components of the adenylate cyclase complex.

A recent study supports the hypothesis that activation of adenylate cyclase by calcitonin is not typical of hormonal stimulation of most forms of adenylate cyclase (15). Lamp et. al report that calctionin activation of adenylate cyclase is persistent in three separate lines of human breast cancer cells. Binding of calcitonin in this system is tight and poorly reversible. The results of these workers were interpreted thus: calcitonin led to an enhanced association of the regulatory and catalytic components of adenylate cyclase.

Our results for both the porcine and human renal cortical adenylate cyclase would also suggest that the calcitonin-stimulated adenylate cyclase is capable of a greater degree of association than other hormone-responsive adenylate cyclases. Hence, this hormonal stimulation survives detergent treatment whereas most others do not. In our system the association is induced by NaF, whereas in the study on human breast cancer cells calcitonin itself induced the association. We have not tried solubilizing with calcitonin, but not NaF, present.

The fact that the glucagon response also solubilizes with the human renal cortical adenylate cyclase suggests that this system may also show enhanced interactions of components. This question remains to be resolved by directly comparing the interactions of glucagon-stimulated adenylate cyclase components from porcine kidneys to those of human kidneys.

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