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PREPARATION OF RENAL CORTEX BASAL-LATERAL AND BRUSH BORDER MEMBRANES

LOCALIZATION OF ADENYLATE CYCLASE AND GUANYLATE CYCLASE ACTIVITIES

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Summary

Luminal brush border and contraluminal basal-lateral segments of the plasma membrane from the same kidney cortex were prepared. The brush border membrane preparation was enriched in trehalase and γ-glutamyltranspeptidase, whereas the basal-lateral membrane preparation was enriched in $(Na^+ + K^+)$-ATPase. However, the specific activity of $(Na^+ + K^+)$-ATPase in brush border membranes also increased relative to that in the crude plasma membrane fraction, suggesting that $(Na^+ + K^+)$-ATPase may be an intrinsic constituent of the renal brush border membrane in addition to being prevalent in the basal-lateral membrane. Adenylate cyclase had the same distribution pattern as $(Na^+ + K^+)$-ATPase, i.e higher specific activity in basal-lateral membranes and present in brush border membranes. Adenylate cyclase in both membrane preparations was stimulated by parathyroid hormone, calcitonin, epinephrine, prostaglandins and 5′-guanylylimidodiphosphate. When the agonists were used in combination enhancements were additive. In contrast to the distribution of adenylate cyclase, guanylate cyclase was found in the cytosol and in basal-lateral membranes with a maximal specific activity (NaN_3 plus Triton X-100) 10-fold that in brush border membranes. ATP enhanced guanylate cyclase activity only in basal-lateral membranes. It is proposed that guanylate cyclase, in addition to $(Na^+ + K^+)$-ATPase, be used as an enzyme "marker" for the renal basal-lateral membrane.

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Abbreviations: cyclic AMP, adenosine 3′,5′-monophosphate; cyclic GMP, guanosine 3′,5′-monophosphate; GMP-PNP, 5′-guanylylimidodiphosphate.

Introduction

The renal proximal tubule cell, which transports solutes vectorially across the tubular epithelium, is characterized by a plasma membrane that is differentiated into two distinct segments, the luminal brush border and the contraluminal basal-lateral membranes. The asymmetry is manifest from findings that the two membranes differ ultrastructurally, in chemical composition, enzymically, and functionally [1,2]. A variety of methods have been reported for isolating either the renal brush border or the basal-lateral membrane, and recently these procedures have been reviewed and compared [3]. It is generally agreed that the brush border membrane is enriched in the disaccharidases trehalase and maltase, γ-glutamyltranspeptidase, and alkaline phosphatase [4–8], whereas the basal-lateral membrane is enriched in $(Na^+ + K^+)$-ATPase [9—11], and that these enzymes serve as "markers" in evaluating preparations. With one exception, the two membranes have been purified by separate procedures starting with different kidneys. Only by free-flow electrophoresis has a common crude plasma membrane fraction been dissociated into its brush border and basal-lateral components [12]. However, the general applicability of this method is limited because the special instrumentation is not ordinarily available and the need to pool kidneys from several animals, e.g. 20 rats, for one electrophoresis run. In the present study, a differential centrifugation and continuous sucrose density gradient method is described for the preparation of basal-lateral and brush border membranes from a single animal.

The polarity of the renal tubule cell is also a factor in the hormonal regulation of transepithelial transport. The precise localization of adenylate cyclase, which is found in the plasma membrane of many different cell types, is a specific problem in the asymmetric plasma membrane of the renal epithelial cell. Preparations of basal-lateral membranes show comparable enrichments in $(Na^+ + K^+)$-ATPase activity and hormone-sensitive adenylate cyclase [9,13]. This membrane, from rat kidney, also possesses high-affinity receptor sites for salmon calcitonin [14]. However, similar membrane preparations from other species, i.e. man, dog, and cow, do not have binding sites for this calcitonin and contain little, if any, calcitonin-sensitive adenylate cyclase [14]. The presence of adenylate cyclase in the brush border membrane region has not been ruled out. A 2—3-fold increase in specific activities of basal and hormone-stimulated adenylate cyclase in brush border membranes relative to homogenates has been reported [8]. Brush border membranes also possess high-affinity calcitonin binding sites and have more receptors per mg of protein than do the basal-lateral membranes [15]. Parathyroid hormone receptors are also found on both membranes [13]. Moreover histochemical studies suggest that F^-- and parathyroid hormone-activated adenylate cyclase is localized predominantly in the brush border membrane with little, if any, reaction precipitate seen in the basal-lateral infoldings [16,17]. The localization of guanylate cyclase in the kidney is essentially unknown. Some activity is found in the supernatant fraction, with additional activity being located in undefined particulate fractions [18]. Thus, the localizations of the two nucleotide cyclases are equivocal. The simultaneous preparation of basal-lateral and brush border membranes from a common crude plasma membrane fraction permits a further examination of this question.

Materials and Methods

Preparation of membranes

Crude plasma membranes were prepared from the renal cortex of New Zealand white male rabbits by a modification of the procedures of Marx et al. [9]. The excised kidneys were quickly chilled in ice-cold medium comprised of 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris · HCl buffer, pH 7.5 (medium A). All subsequent steps of the procedure were carried out in a coldroom (2—4°C) or on ice. The kidneys were decapsulated; the cortices were dissected and then thoroughly minced. The minced tissue (10—15 g wet weight from one animal) was homogenized in medium A, using a ratio of 1 g of tissue to 3 ml of medium, with nine strokes of a loosely fitting Dounce homogenizer. The homogenate was centrifuged rapidly and briefly by setting the centrifuge to attain 2400 × *g* and when this force was reached stopping the centrifuge immediately. The residue was discarded and the supernatant was recentrifuged in the same manner. The centrifugation procedure was repeated a third time. The supernatant was then centrifuged at 2400 × *g* for 15 min. The resulting supernatant was discarded. The fluffy upper portion of the sediment was sloughed from the pellet by gentle swirling with about 2 ml of medium A. This loosely packed particulate fraction was resuspended with a Dounce homogenizer and it represented the crude plasma membrane preparation.

The crude plasma membrane suspension (about 2 ml) was layered onto a 30 ml continuous gradient of 32.5—41.5% (w/w) sucrose dissolved in 10 mM Tris · HCl buffer, pH 7.5, containing 1 mM EDTA (medium B). The gradient was centrifuged at 105 000 × *g* for 1 h. Fractions (1.5 ml) were collected from the bottom of the centrifuge tube and diluted with one-half volume of medium B. The membranes in each fraction were sedimented by centrifugation at 30 000 × *g* for 15 min and the resulting pellet resuspended in 2 mM Tris · HCl buffer, pH 7.5, containing 0.2 mM EDTA. The membranes were assayed for γ-glutamyltranspeptidase and trehalase activities, to indicate fractions enriched in brush border membranes, and for $(Na^+ + K^+)$-ATPase activity, to indicate fractions enriched in basal-lateral membranes [1,2]. The fractions corresponding to brush border or basal-lateral membranes were pooled. The consolidated fractions were then diluted with one-half volume of medium B and the membranes sedimented by centrifugation at 30 000 × *g* for 15 min.

The consolidated partially purified preparation of brush border membranes was suspended in medium A and centrifuged at 30 000 × *g* for 10 min. The supernatant was discarded. The white loosely packed portion of the pellet, containing the brush border membranes, overlying the brownish tightly packed core, containing primarily mitochondria, was collected. The brush border membranes were washed by this procedure three additional times. After each centrifugation, the supernatant was discarded and the fluffy membranes were removed from the diminishing, densely packed, contaminating portion of the pellet. The final sediment of purified brush border membranes was suspended in 2 mM Tris · HCl buffer, pH 7.5, containing 0.2 mM EDTA.

The consolidated partially purified preparation of basal-lateral membranes was resuspended in 2 ml of medium A and the suspension was subjected to a second continuous sucrose gradient centrifugation as described above. Frac-

tions representing basal-lateral membranes, as indicated by ($Na^+ + K^+$)-ATPase activity, were pooled and the membranes sedimented by centrifugation. The basal-lateral membranes were resuspended in medium A and the preparation was further purified by three washings identical to those for purifying the brush border membranes. Since the distributions of marker enzyme activities after continuous sucrose gradient centrifugations were found to be very consistent, in later experiments the fractions corresponding to brush border and basal-lateral membranes were routinely pooled without assay. However, purity of the final preparations was always monitored by determining specific activities of their respective marker enzymes.

Assays

Trehalase and ($Na^+ + K^+$)-ATPase activities were measured as described [4]. Previously reported methods were used for the assays of γ-glutamyltranspeptidase [19], glucose-6-phosphatase [9], succinate dehydrogenase [20], and lactate dehydrogenase [21] activities. ATP : NMN transferase activity was measured as described [22], with the NAD formed determined by coupling with alcohol dehydrogenase [23]. Acid phosphatase activity was assayed by the method of Rothstein and Blum [24], except that the incubation was at 30°C and 0.2 M NaOH was used to terminate the reaction. Protein was determined by a standard procedure [25].

Adenylate cyclase activity was assayed in a 50 μl reaction mixture containing 20 mM Tris · HCl buffer, pH 7.5, 5 mM $MgCl_2$, 1 mM [α-^{32}P]ATP (approx. 1 μCi), 2 mM theophylline, 2 mM cyclic AMP, 20 mM creatine phosphate, 25 μg creatine phosphate kinase, and 10—15 μg of membrane protein. The incubation period was 30 min and the temperature 30°C. The reaction was terminated by the addition of 0.1 ml stopping solution comprised of 5 mM Tris · HCl, pH 7.5, 40 mM ATP, 1.4 mM cyclic AMP, and 2% sodium dodecyl sulfate, followed by heating in a water bath at 85—100°C for 2 min. Prior to heating, 50 μl of cyclic [8-^{3}H]AMP (10 nCi) was added to the mixture to monitor recovery. After heating, the total volume was made to 1 ml with 5 mM Tris · HCl, pH 7.5. Cyclic [^{32}P]AMP formed was eluted by a combined column chromatography procedure on Bio-Rad AG 50W-X8 and neutral alumina, as described by Salomon et al. [26]. Radioactivity was measured in Aquasol (New England Nuclear) in a Packard Tri-Carb liquid scintillation counter. Adenylate cyclase reaction was linear with time for at least 1 h and with protein to at least 32 μg of membrane protein per assay for basal and NaF-stimulated activities.

Guanylate cyclase activity was assayed in a 50 μl reaction mixture containing 50 mM Tris · HCl buffer, pH 7.5, 2 mM $MnCl_2$, 0.4 mM [α-^{32}P]GTP (approx. 0.4 μCi), 5 mM theophylline, 1 mM cyclic GMP, 10 mM creatine phosphate, 12.5 μg creatine phosphate kinase, and 10—20 μg of membrane protein. The incubation period was 30 min and the temperature was 30°C. The reaction was terminated by the addition of 0.1 ml ice-cold diluting solution consisting of 50 mM Tris · HCl buffer, pH 7.5, 0.4 mM GTP, and 0.5 mM cyclic GMP, followed immediately by heating in a boiling water bath for 2 min. Cyclic [8-^{3}H]GMP (10 nCi) was added prior to heating to evaluate recovery. After heating, the total volume was made to 1 ml with 5 mM Tris · HCl buffer, pH 7.5. Cyclic [^{32}P]GMP was eluted by a two-column chromatography procedure modified

from that described by Nesbitt et al. [27]. In the present study, the reaction mixture was poured onto a 1 cm column comprised of 2 ml Bio-Rad AG 50W-X8 resin. The column was washed with 1 ml of H_2O, the eluate being discarded. Cyclic GMP was eluted from the ion-exchange column with 2 ml of H_2O onto a 1 cm column of alumina (1 g). The alumina column was washed with 3 ml of 0.1 M imidazole · HCl buffer, pH 7.5, the eluate being discarded. Cyclic GMP was eluted with the addition of 5 ml of the imidazole buffer. Radioactivity was measured in ACS (Amersham/Searle) in a Packard Tri-Carb liquid scintillation counter. The guanylate cyclase reaction was linear with time for at least 30 min and with protein for at least 25 μg per assay.

Materials

Cyclic AMP, AMP, NADH, and GTP were purchased from P-L Biochemicals. Phosphocreatine kinase, glucose-6-phosphate dehydrogenase, hexokinase, NAD, NADP, and phosphoenolpyruvate were obtained from Boehringer. ATP, cyclic GMP, NMN, phosphocreatine, *p*-nitrophenyl phosphate and neutral alumina were from Sigma. GMP-PNP was purchased from I.C.N. Pharmaceuticals. [α-^{32}P]ATP (50—160 Ci/mmol) and [α-^{32}P]GTP (2—20 Ci/mmol) were obtained from New England Nuclear. Cyclic [8-^{3}H]AMP (27 Ci/mmol) and cyclic [8-^{3}H]GMP (10—30 Ci/mmol) were from Amersham/Searle.

Epinephrine, DL-propranolol, and isoproteronol were obtained from Sigma. Synthetic vasopressin and insulin were from Calbiochem and parathyroid hormone 1-34 was from Beckman. Synthetic salmon calcitonin was a gift from Armour Pharmaceuticals and prostaglandins A_1, E_1, and $F_{2\alpha}$ were donated by Upjohn Co.

Results

Isolation of basal-lateral and brush border membranes

The crude plasma membrane fraction, prepared as described in Methods and Materials, comprised approx. 5% of the total protein of the renal cortex homogenate and had about a 3-fold increase in specific activities for the "marker" enzymes trehalase, γ-glutamyltranspeptidase and ($Na^+ + K^+$)-ATPase relative to those in the homogenate. Fig. 1A illustrates the distribution of the three enzymes in fractions obtained after centrifugating the crude plasma membrane preparation on a continuous sucrose gradient. The profile indicates that the bulk of the trehalase and γ-glutamyltranspeptidase activities were in fractions 3—10, suggesting the presence therein of the brush border membrane. The profile of ($Na^+ + K^+$)-ATPase activity was less focused and appeared to be distributed in two general regions. One region superimposed with that for trehalase and γ-glutamyltranspeptidase. The other region, comprising fractions 11—19, was relatively low in brush border membrane enzymes and presumably contained the basal-lateral membranes. Fractions 3—10, corresponding to the brush border membranes, were combined and the membranes further purified as described in Methods and Materials. Pooled fractions 11—19, corresponding to the basal-lateral membranes, were subjected to a second centrifugation on the sucrose gradient. As shown in Fig. 1B, ($Na^+ + K^+$)-ATPase activity now clearly separated from most of the contaminating trehalase and γ-glutamyltranspepti-

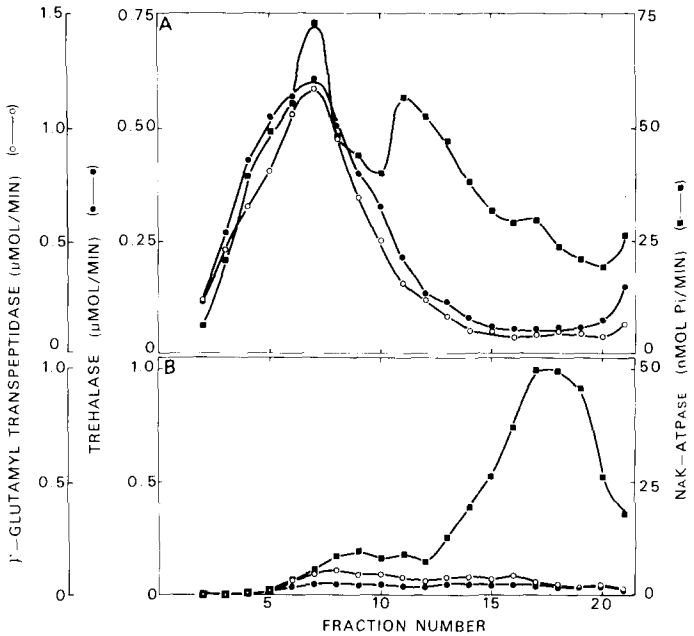

Fig. 1. Distributions of trehalase, γ-glutamyltranspeptidase and (Na^+ + K^+)-ATPase activities in fractions obtained after centrifugation of crude plasma membranes on a continuous sucrose gradient, described in the text. (A) Represents the distributions after the initial fractionation. (B) Represents the distributions of enzymes in pooled fractions 11—19 after a second centrifugation on the gradient. Activities are expressed as total activity per fraction.

dase activities. Fractions 14—21, corresponding to the basal-lateral membranes, were combined and the membranes further purified as described in Methods and Materials.

Evaluation of the purity of the membrane preparations

Separation of the basal-lateral from brush border membranes was assessed biochemically by the activities of "marker" enzymes and this is reported, in part, in Table I. The purified preparation of basal-lateral membranes contained 1.5% of the total protein of the crude plasma membrane. The specific activity of (Na^+ + K^+)-ATPase in the basal-lateral membrane increased 6-fold relative to that in the crude plasma membrane. The recovery of (Na^+ + K^+)-ATPase was 10%. In contrast, the specific activities of trehalase and γ-glutamyltranspeptidase decreased by factors of about 0.7, constituting recoveries of 1%. The biochemical purity of the basal-lateral membrane preparation was also evaluated by assays of other enzymes (not shown). For example, lactate dehydrogenase and ATP : NMN adenylyltransferase activities were not measurable, suggesting little, if any, contamination by the cytosol or nuclei. Some contamination of the basal-lateral membrane preparation by mitochondria, lysosomes and endoplasmic reticulum was indicated, as the specific activities of succinate dehydrogenase (Table I), acid phosphatase and glucose-6-phosphatase (not shown) were not appreciably decreased in the purified membrane preparation relative to those in the crude plasma membrane fraction. The succinate dehydrogenase specific

TABLE I

SPECIFIC ACTIVITIES OF ENZYMES IN PREPARATIONS OF THE RENAL CORTEX BASAL-LATERAL AND BRUSH BORDER MEMBRANES

Each datum represents the mean ± S.E. for at least seven preparations.

Enzyme	Crude plasma membrane	Basal-lateral membrane	Brush border membrane
$(Na^+ + K^+)$-ATPase [a]	3.32 ± 0.78	19.9 ± 0.7	8.4 ± 1.1
Trehalase [b]	28.9 ± 5.2	18.7 ± 2.9	116.0 ± 22.0
γ-Glutamyltranspeptidase [c]	92.0 ± 7.5	65.0 ± 6.8	356.0 ± 22.0
Succinate dehydrogenase [d]	9.6 ± 2.2	9.2 ± 0.6	1.4 ± 0.5
Protein [e]	49.0 ± 1.0	0.76 ± 0.07	1.91 ± 0.17

[a] μmol inorganic phosphate produced/h per mg protein
[b] μmol glucose produced/h per mg protein.
[c] μmol glutamyl residues transferred/h per mg protein.
[d] μmol succinate oxidized/h per mg protein.
[e] mg.

activity was 9.2 μmol/h per mg protein, seemingly suggesting that mitochondrial contamination was substantial. Indeed, the specific activity of the enzyme in renal cortex mitochondria was 21.4 μmol/h per mg protein, perhaps inferring that mitochondria comprised 40% of the basal-lateral membrane preparation. However, this percentage probably was grossly overestimated. Succinate dehydrogenase was localized exclusively in the mitochondrial inner membrane [28]; hence the specific activity of the enzyme in intact mitochondria would be significantly lower than in inner membranes, in the absence of mitochondrial matrix protein and outer membranes. Electron micrographs of the basal-lateral membrane preparation revealed the presence predominantly of mitochondrial fragments with few intact organelles (Fig. 2). Moreover, succinate dehydrogenase : monoamine oxidase ratios, the latter enzyme an outer membrane "marker" [28], was increased over 50% in the basal-lateral membrane preparation relative to the ratio in intact mitochondria, indicating an enrichment of the mitochondrial inner membrane in the basal-lateral membrane preparation. These findings suggested that the true level of contamination of the basal-lateral membrane preparation by mitochondria, although significant, was considerably less than the 40% level.

The purified preparation of brush border membranes contained 4% of the total protein of the crude plasma membrane fraction. The specific activities of trehalase and γ-glutamyltranspeptidase in the brush border membrane preparation were increased 4-fold relative to those in the crude plasma membrane preparation. Recoveries were 16%. The relative specific activity of $(Na^+ + K^+)$-ATPase in the preparation also increased, by a factor of 2.5. The recovery of $(Na^+ + K^+)$-ATPase was only 1%, however. Thus, the specific activity of trehalase in the brush border membrane was 6.2 times that in the basal-lateral membrane preparation, whereas $(Na^+ + K^+)$-ATPase was enriched 2.4 times in the basal-lateral relative to the brush border membrane preparations. Succinate dehydrogenase specific activity in the brush border membrane preparation was decreased to one-seventh that in the crude plasma membrane fraction. The purified brush border membranes prepared in this study were not evaluated for

contamination by cytosol, nuclei, lysosomes or microsomes since other preparations having comparable trehalase and γ-glutamyltranspeptidase specific activities contained little, if any, contamination by these cell components [4].

Electron microscopic evaluation of the purified brush border and basal-lateral membrane preparations are shown in Fig. 2. The brush border membrane preparation was homogeneous with only rare contamination by other membranous cell constituents. The entire apical (luminal) segment of the plasma membrane was isolated, with finger-like microvilli projections still largely attached. Occasional tight junctions were visible, representing the juxtaposition of brush border membranes of adjoining proximal tubule cells. The basal-lateral membrane preparation appeared less homogeneous, confirming the biochemical evaluation. Numerous closed and open vesicles derived from the contraluminal segment of the plasma membrane were seen. Also visible were tight junctions and membrane pairs with interspaces, resembling the infolded basal-lateral membranes in situ. Other cell particulates recognizable as disrupted mitochondria were present in moderate number, however.

Adenylate cyclase

The specific activities of basal and F^--stimulated adenylate cyclase in the basal-lateral and brush border membrane preparations are shown in Table II. The specific activity of the F^--stimulated enzyme was enriched 3.6-fold in the basal-lateral membrane preparation relative to that in the crude plasma membrane fraction. The specific activity of the F^--stimulated enzyme in the brush border membrane was also increased, but only 1.6-fold. To be noted, however, was that the distribution of adenylate cyclase between the two membrane preparations was essentially identical with that for $(Na^+ + K^+)$-ATPase. This was evident from the ratios of units of adenylate cyclase: $(Na^+ + K^+)$-ATPase activities, which for the basal-lateral membrane was 425 : 20, a value of 21.4, and for the brush border membrane was 190 : 8.4, a value of 22.7.

Table III shows the effects of different hormones and GMP-PNP on adenylate cyclase activity in the basal-lateral membrane. GMP-PNP stimulated activity 5-fold, compared to a 7-fold increase with F^-. Notable stimulations were obtained with parathyroid hormone, calcitonin and prostaglandins E_1, A_1, and $F_{2\alpha}$. Epinephrine significantly increased the basal-lateral membrane adenylate

TABLE II

SPECIFIC ACTIVITIES OF ADENYLATE CYCLASE IN PREPARATIONS OF THE RENAL CORTEX BASAL-LATERAL AND BRUSH BORDER MEMBRANES

Reaction conditions were as described in the text with the NaF concentration when added being 7 μM. Each datum represents the mean ± S.E. for four experiments.

Preparation	Adenylate cyclase (pmol/min per mg protein)	
	Basal	+ NaF
Crude plasma membrane	24.9 ± 5.5	118 ± 11
Basal-lateral membrane	48.5 ± 4.7	425 ± 51
Brush border membrane	22.9 ± 3.0	190 ± 14

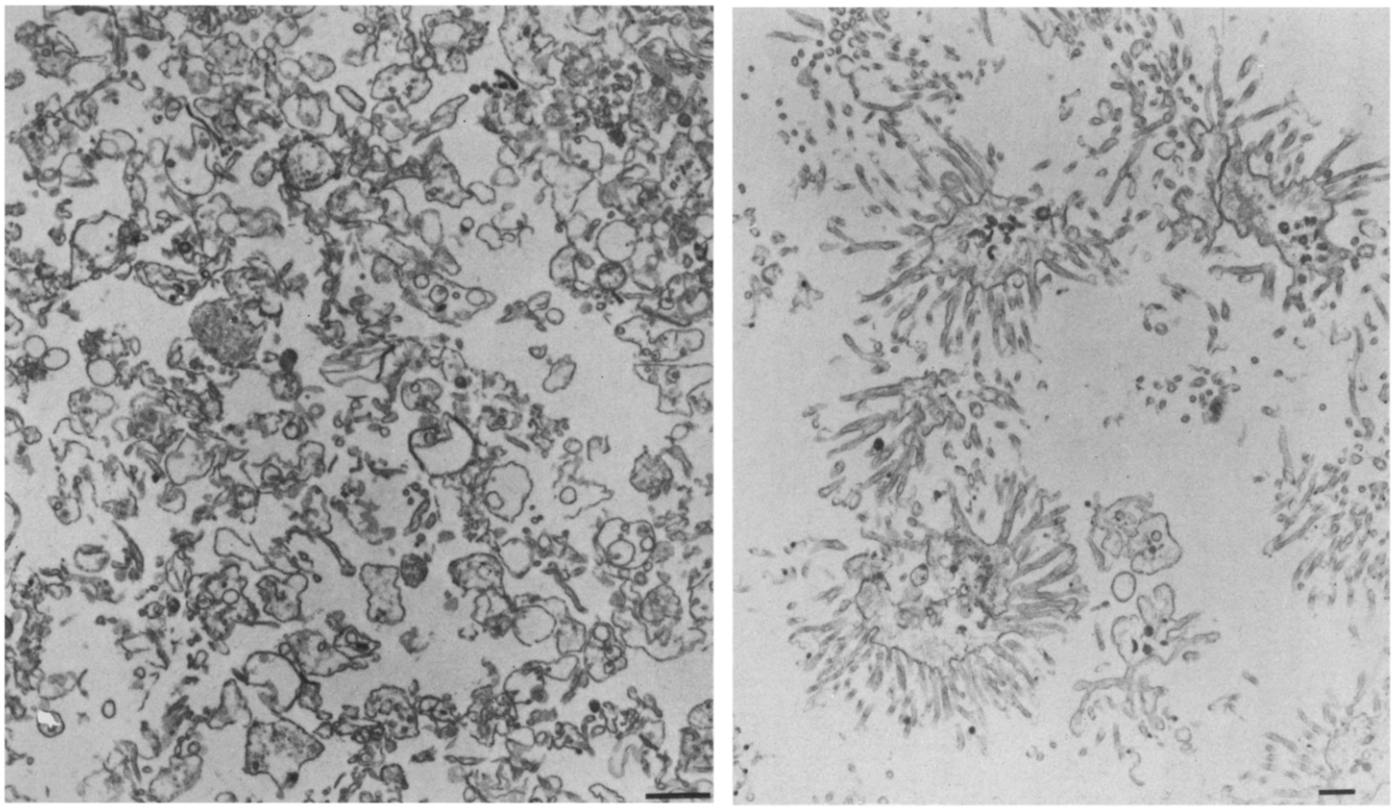

Fig. 2. Electron micrographs of the renal cortex brush border (right) and basal-lateral (left) membrane preparations. Bar indicates 1 μm.

TABLE III

EFFECT OF HORMONES ON THE RENAL CORTEX BASAL-LATERAL MEMBRANE ADENYLATE CYCLASE

The basal adenylate activities in experiments I and II were 43.9 ± 7.8 and 43.3 ± 5.6 pmol of cyclic AMP produced/min per mg of membrane protein, respectively. These values are designated as having a relative activity of 1.00. The concentrations of effectors were $7 \cdot 10^{-6}$ M NaF, $5 \cdot 10^{-6}$ M GMP-PNP, 75 units/ml parathyroid hormone 1-34, 10 μg/ml calcitonin, 10 μg/ml prostaglandin E_1, 10 μg/ml prostaglandin A_1, 10 μg/ml prostaglandin $F_{2\alpha}$, 2 μg/ml vasopressin, $6 \cdot 10^{-5}$ M epinephrine, and $1 \cdot 10^{-4}$ propranolol. Each datum represents the mean ± S.E. of at least three experiments.

Experiment	Relative activity
I. Basal	1.00
NaF	6.95 ± 0.29
GMP-PNP	5.32 ± 0.34
Parathyroid hormone 1-34	5.03 ± 0.71
Calcitonin	2.69 ± 0.57
Prostaglandin E_1	2.79 ± 0.17
Prostaglandin A_1	1.79 ± 0.13
Prostaglandin $F_{2\alpha}$	1.31 ± 0.04
Vasopressin	1.45 ± 0.17
Epinephrine	1.29 ± 0.06
Propranolol	1.06 ± 0.03
Epinephrine + propranolol	0.87 ± 0.10
GMP-PNP + parathyroid hormone 1-34	8.50 ± 0.54
GMP-PNP + calcitonin	6.72 ± 0.67
II. Basal	1.00
GMP-PNP	5.32 ± 0.34
Parathyroid hormone 1-34	4.27 ± 0.44
Calcitonin	2.83 ± 0.88
Prostaglandin E_1	1.90 ± 0.13
Prostaglandin E_1 + GMP-PNP	7.32 ± 0.76
Prostaglandin E_1 + parathyroid hormone 1-34	4.93 ± 0.43
Prostaglandin E_1 + calcitonin	3.82 ± 0.88

cyclase and this increase was completely blocked by the β-receptor antagonist propranolol. Vasopressin affected a slight stimulation, although the possible contamination of undetermined magnitude of the basal-lateral membrane preparation with the vasopressin-sensitive adenylate cyclase in plasma membranes from the distal tubules in the renal cortex [9] was not ruled out. Insulin (1.2 munits/ml) did not affect adenylate cyclase activity (not shown). The concentration of each agonist shown in Table III was at or very close to that needed to achieve maximum activation. When the agonists were used in combination stimulations were additive. Thus, GMP-PNP plus parathyroid hormone, calcitonin or prostaglandin E_1 affected increases in adenylate cyclase activity generally equal to the sum of the increases produced by the individual agonist. Combinations of hormones, i.e. prostaglandin E_1 plus parathyroid hormone or calcitonin, also showed additive activations. No differences, qualitatively or quantitatively, were found between the actions of these agonists on adenylate cyclase in the basal-lateral and brush border membrane preparations.

Guanylate cyclase

The distribution of guanylate cyclase in the renal cortex was different from

TABLE IV

SPECIFIC ACTIVITIES OF GUANYLATE CYCLASE IN FRACTIONS OF THE RENAL CORTEX

Reaction conditions were as described in the text. The enzyme was stimulated by the addition of 0.4 mM NaN_3 to the reaction mixture and by pretreatment of the preparations with 1% Triton X-100. The final concentration of detergent in the incubation mixture was 0.4%. The cytosol fraction was obtained by centrifuging the homogenate at 105 000 × *g* for 1 h. Each datum represents the mean ± S.E. for at least five experiments.

Preparation	Guanylate cyclase (pmol/min per mg protein)	
	Basal	Stimulated
Homogenate	6.4 ± 0.3	57 ± 5
Basal-lateral membrane	3.9 ± 1.0	311 ± 15
Brush border membrane	3.1 ± 0.5	28 ± 4
Cytosol	12.8 ± 1.3	38 ± 4

that of adenylate cyclase. As shown in Table IV, guanylate cyclase activity was found in the cytosol as well as in the basal-lateral and brush border membrane preparations. The specific activities of the unstimulated enzyme in the two membranes were similar. Previous studies, however, showed that guanylate cyclase activity was latent and could be stimulated by NaN_3 and Triton X-100 [29]. When the renal preparations were treated with these effectors, activities in the cytosol and brush border membrane preparation were appreciably increased, but the activity in the basal-lateral membrane preparation was greatly stimulated, at least 50-fold. In fact, the specific activity of the azide plus detergent-stimulated guanylate cyclase in basal-lateral membranes was 10 times that in the brush border membrane. Moreover, the specific activity of the fully expressed guanylate cyclase in the basal-lateral membrane approached that of the F^--stimulated adenylate cyclase. The guanylate cyclase activity in the basal-lateral membrane was additionally distinguished from the activities in the brush border membrane and cytosol by the action of ATP. ATP was reported to enhance particulate bound guanylate cyclase in calf uterus [30]. Table V shows that 100 μM ATP doubled the activity of the enzyme in the basal-lateral mem-

TABLE V

EFFECT OF ATP ON GUANYLATE CYCLASE ACTIVITIES IN DIFFERENT FRACTIONS OF THE RENAL CORTEX

Reaction conditions were as described in text with 100 μM ATP added, when indicated. Each datum represents the mean ± S.E. for the number of experiments shown in parentheses.

Preparation	ATP	Activity (pmol/min per mg protein)
Basal-lateral	—	6.9 ± 1.6 (4)
	+	14.7 ± 2.4 (4)
Brush border membrane	—	6.0 ± 1.2 (4)
	+	5.5 ± 0.9 (4)
Cytosol	—	6.8 ± 0.7 (2)
	+	4.4 ± 0.1 (2)

brane of the renal tubule. In contrast, guanylate cyclase activities in the brush border membrane as well as in the cytosol were not activated and, if anything, were inhibited. Thus, these results confirmed the activation of membrane-bound guanylate cyclase by ATP and showed in addition that in the kidney activation was restricted to the enzyme in the basal-lateral membrane.

Discussion

Luminal brush border and contraluminal basal-lateral segments of the plasma membrane of the proximal tubule cell from the same kidney were prepared concurrently and were partially characterized. In the brush border membrane preparation, specific activities of trehalase and γ-glutamyltranspeptidase were increased 4-fold relative to those in the crude plasma membrane fraction and 12—16-fold relative to those in the homogenate of the cortex. These enhancements are in accord with the 12—18-fold increases in specific activities of the two enzymes, when this membrane was isolated independently [4—6]. These increments may also be compared to enrichments of 1.3- and 10-fold for alkaline phosphatase in brush border membranes relative to specific activities in unfractionated plasma membranes and cortical homogenates, respectively, when brush border and basal-lateral membranes were partitioned by free-flow electrophoresis [31]. The specific activity of trehalase in the brush border membrane preparation was over 6 times that in the basal-lateral membrane preparation. This agrees with the 5-fold increase in specific activity of alkaline phosphatase in brush border relative to that in basal-lateral membranes, when the two membranes were separated by the earlier procedure [13]. In other studies (Slack, E., Liang, C.T. and Sacktor, B., unpublished observations), it was found that the Na^+ gradient-dependent sugar and amino acid transport systems in the brush border membranes prepared by the present method were as active and competent as those reported in other brush border membranes [32,33]. Thus, it is concluded that the brush border membranes isolated by the present procedure meet or exceed other preparations in quality.

The specific activities of $(Na^+ + K^+)$-ATPase in the basal-lateral membranes prepared by this method and by free-flow electrophoresis [13,31] were similar. With other procedures designed to isolate only basal-lateral membranes, specific activities half [9] or 4 times greater [11] than described here were obtained. $(Na^+ + K^+)$-ATPase specific activity was only 2.4-fold higher in the basal-lateral than in the brush border membrane preparations. It can be calculated that as much of the total enzyme activity was recovered in the brush border as in the basal-lateral membrane preparation, although differential loss in the yield of one membrane cannot be ruled out. However, considering that the microvilli of the brush border increase the luminal surface of the tubule cell approx. 40 times [3], it appears that a significant proportion of the total plasma membrane $(Na^+ + K^+)$-ATPase may be found in the brush border segment. When the membranes were separated by free-flow electrophoresis [13], the contraluminal membranes had a $(Na^+ + K^+)$-ATPase specific activity 4.6-fold that in the luminal membranes, but data on membrane yields and enzyme recoveries were not reported; therefore, distribution of enzyme activity in the two preparations could not be determined. Nevertheless, it was argued that $(Na^+ + K^+)$-ATPase

activity was localized exclusively in basal-lateral membranes and that any activity found in brush border membrane preparations reflected contamination by basal-lateral membranes [12,13]. On the other hand, the present findings favor the view that the complete localization of $(Na^+ + K^+)$-ATPase remains unresolved and that in addition to its prominence in basal-lateral membranes the enzyme may be an intrinsic constituent of the renal brush border. Support for this latter view comes from the following observations. (1) If it is assumed that there is no $(Na^+ + K^+)$-ATPase activity in the brush border membrane, then the finding that the specific activity of the enzyme in the basal-lateral was 2.4 times greater than in the brush border membrane infers that the brush border membrane preparation was 40% contaminated by basal-lateral membranes. Electron micrographs (Fig. 2), however, show little contamination with only occasional tags of basal-lateral membranes, i.e. the tight junctions, evident. (2) The specific activity of the fully stimulated guanylate cyclase (Table IV) in the basal-lateral membranes was 10-fold that in the brush border membrane, indicating that brush border membrane preparation was contaminated by basal-lateral membranes maximally to 10%. That it was probably less than 10% was indicated by the finding that ATP did not stimulate the enzyme in the brush border membrane preparation (Table V). This conclusion is supported additionally by studies, reported elsewhere [34], in which cyclic AMP phosphodiesterase activities in the basal-lateral and brush border membranes were compared. Cyclic AMP phosphodiesterase, which was stimulated by cyclic GMP plus ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*'-tetraacetic acid (EGTA), was found in the basal-lateral membrane, whereas only 9% of this stimulatable activity was in the brush border membrane. The differences between the ratio of $(Na^+ + K^+)$-ATPase activity in the basal-lateral membrane relative to that in the brush border membrane and the ratios of guanylate cyclase and cyclic GMP-sensitive cyclic AMP phosphodiesterase activities in the two membrane preparations suggest that $(Na^+ + K^+)$-ATPase activity is present in the brush border membrane. As a corollary to this sugestion, it is proposed that, when only membranes are being compared, that maximally stimulated guanylate cyclase and cyclic GMP-activated cyclic AMP phosphodiesterase be used in addition to $(Na^+ + K^+)$-ATPase as enzyme "markers" for the renal basal-lateral membrane.

In agreement with earlier reports [9,13], adenylate cyclase had the same distribution pattern as $(Na^+ + K^+)$-ATPase. Significant adenylate cyclase activity was associated with the brush border membrane preparation, however. In addition, relative stimulations of the enzyme by hormones, i.e. parathyroid hormone, calcitonin, prostaglandins and epinephrine, were the same in the basal-lateral and brush border membrane preparations. This is in accord with the demonstration of hormone receptors on the two membranes [13,15]. The finding of adenylate cyclase in the basal-lateral membrane negates the failure to detect the enzyme in this membrane by histochemical techniques [16,17]. The histochemical detection of adenylate cyclase is subject to various pitfalls, which may not have been fully appreciated [35]. In contrast to adenylate cyclase, guanylate cyclase when fully exposed was found predominantly in the basal-lateral membrane (Table IV). The physiological significance of this active guanylate cyclase in the renal tubule basal-lateral membrane remains to be established.

The present findings substantiate further that the brush border and basal-lateral membranes have distinct enzymic compositions. The membrane also differ in their transport properties (ref. 31, and Slack, E., Liang, C.T. and Sacktor, B., unpublished observations). Since tubular absorption requires transport of solutes across both membranes, control of the overall process may occur at each site by different mechanisms. The procedure described in this paper to isolate concurrently both membranes from the same kidney and the demonstration of specific localizations for enzymes determining cyclic nucleotide metabolism should facilitate further studies on the regulation of renal transport.

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References

1 Sacktor, B. (1977) in Current Topics in Bioenergetics (Sanadi, R., ed.) Vol. 6, pp. 39—81 Academic Press, New York
2 Kinne, R. (1976) in Current Topics in Membranes and Transport (Bronner, F. and Kleinzeller, A., eds.), Vol. 8, pp. 209—267
3 Sacktor, B. (1977) in Mammalian Cell Membranes (Jamieson, G.A. and Robinson, D.M., eds.), Vol. 4, Butterworths, London, in the press
4 Berger, S.J. and Sacktor, B. (1970) J. Cell Biol. 47, 637—645
5 Quirk, S.J. and Robinson, G.B. (1972) Biochem. J. 128, 1319—1328
6 George, S.G. and Kenny, A.J. (1973) Biochem. J. 134, 43—57
7 Kinne, R. and Kinne-Saffran, E. (1969) Pfluegers. Arch. 308, 1—15
8 Wilfong, R.F. and Neville, Jr., D.M. (1970) J. Biol. Chem. 245, 6106—6112
9 Marx, S.J., Woodard, C.J. and Aurbach, G.D. (1972) Science 178, 999—1001
10 Schmidt, U. and Dubach, U.C. (1971) Pfluegers Arch. 330, 265—270
11 Ebel, H., Aulbert, E. and Merker, H.J. (1976) Biochim. Biophys. Acta 433, 531—546
12 Heidrich, H.G., Kinne, R., Kinne-Saffran, E. and Hannig, K. (1972) J. Cell Biol. 54, 232—245
13 Shlatz, L.J., Schwartz, I.L., Kinne-Saffran, E. and Kinne, R. (1975) J. Membrane Biol. 24, 131—144
14 Marx, S.J. and Aurbach, G.D. (1975) Endocrinology 97, 448—453
15 Marx, S.J., Woodard, C. and Aurbach, G.D. (1973) J. Biol. Chem. 248, 4797—4802
16 Sato, T., Garcia-Bunuel, R. and Brandes, D. (1974) Lab. Invest. 30, 222—229
17 Jande, S.S. and Robert, P. (1974) Histochemistry 40, 323—327
18 Criss, W.E., Murad, F. and Kimura, H. (1976) J. Cyclic Nucleotide Res. 2, 11—19
19 Glossmann, H. and Neville, Jr., D.M. (1972) FEBS Lett. 19, 340—344
20 Bonner, W. (1955) in Methods in Enzymology (Colowick, S.P. and Kaplan, N.O., eds.), Vol. 1, pp. 722—729, Academic Press, New York
21 Kornberg, A. (1955) in Methods in Enzymology (Colowick, S.P. and Kaplan, N.O., eds.), Vol. 1, pp. 441—454, Academic Press, New York
22 Kato, T. and Kurowaka, M. (1976) J. Cell Biol. 32, 649—662
23 Williamson, J.R. and Corkey, B.E. (1969) in Methods in Enzymology (Lowenstein, J.M., ed.), Vol. 13, pp. 435—513, Academic Press, New York
24 Rothstein, T.L. and Blum, J.J. (1973) J. Cell Biol. 57, 630—641
25 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
26 Salomon, Y., Londos, C. and Rodbell, M. (1974) Anal. Biochem. 58, 541—548
27 Nesbitt, III, J.A., Anderson, W.B., Miller, Z., Pastan, I., Russell, T.R. and Gospodarowicz, D. (1976) J. Biol. Chem. 251, 2344—2532
28 Schnaitman, C. and Greenawalt, J.W. (1968) J. Cell Biol. 38, 158—175
29 Kimura, H., Mittal, C.K. and Murad, F. (1975) J. Biol. Chem. 250, 8016—8022
30 Siegel, M.I., Puca, G.A. and Cuatrecasas, P. (1976) Biochim. Biophys. Acta 438, 310—323
31 Kinne, R., Murer, H., Klinne-Saffran, E., Thees, M. and Sachs, G. (1975) J. Membrane Biol. 21, 375—395
32 Aronson, P.S. and Sacktor, B. (1975) J. Biol. Chem. 250, 6032—6039
33 Fass, S.J., Hammerman, M.R. and Sacktor, B. (1977) J. Biol. Chem. 252, 583—590
34 Filburn, C.R., Liang, C.T. and Sacktor, B., submitted
35 Lemay, A. and Jarett, L. (1975) J. Cell Biol. 65, 39—50