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PREPARATION OF PROSTATIC PLASMA MEMBRANES DISTRIBUTION OF (Na^+, K^+)-ATPase AND Mg^{2+}-ATPase IN THE RAT VENTRAL PROSTATE

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SUMMARY

1. A method is described for the isolation of a plasma membrane fraction from rat ventral prostate. This fraction is greatly enriched in (Na^+, K^+)-ATPase but also contains a small amount of Mg^{2+}-ATPase and 5′-nucleotidase activities.

2. The activity ratio ($Mg^{2+}+Na^++K^+/Mg^{2+}$ or Mg^++Na^+) of the plasma membrane (Na^+, K^+)-ATPase was 1.7. The enzyme system specifically requires ATP as substrate and is inhibited by Ca^{2+} or ouabain.

INTRODUCTION

The active uptake of amino acids and sugars by the prostate [1] and the activity of the transport-related enzyme, (Na^+, K^+)-ATPase [2], are androgen dependent [3–5]. The (Na^+, K^+)-ATPase system has been postulated to be a receptor for testosterone [6]. Prostatic adenylate cyclase activity has been reported to be enhanced by androgen treatment [7]. In addition, prolactin has effects on the prostate [8], including stimulation of adenylate cyclase activity, which are independent of androgens [9]. To study these phenomena further, methods were designed for the preparation of plasma membranes from rat ventral prostate using procedures modified from those of Meldolesi et al. [10] and Ahmed and Williams-Ashman [3]. A plasma membrane fraction was obtained which was greatly enriched in (Na^+, K^+)-ATPase but contained low levels of Mg^{2+}-ATPase and 5′-nucleotidase activities.

MATERIALS AND METHODS

Adult male rats of the Sprague-Dawley strain (Goffmore Farms, Westboro, Mass.), weighing 300–400 g, were maintained on constant photoperiod (14 h light and 10 h dark) and fed standard laboratory chow and water ad libitum.

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The ventral prostates from 12–24 animals were blotted, pooled and minced and a 10% (w/v) homogenate was prepared with five strokes of a teflon-glass homogenizer in a medium consisting of 0.25 M mannitol, 25 mM Tris · HCl, and 1 mM EDTA, final pH 7.4 [3]. After this procedure, a clump of non-disrupted prostate (which appeared to be primarily fibromuscular tissue) remained and was discarded. The homogenate was filtered through one layer of nylon stocking and centrifuged at $1080 \times g$ for 10 min. The pellet was resuspended in the homogenizing medium with gentle homogenization (1–2 strokes teflon-glass homogenizer) and recentrifuged. The washed pellet was resuspended in homogenizing medium and 2.0 M sucrose containing 25 mM Tris · HCl, pH 7.4, to a final sucrose concentration of 1.6 M. This was overlaid with homogenizing medium and centrifuged 1.5 h at $65\,000 \times g$ in a SW-25 rotor, Beckman model L-2 ultracentrifuge. The membrane layer on 1.6 M sucrose was removed, diluted with 25 mM Tris · HCl, pH 7.4, layered over a discontinuous gradient of 0.6, 1.2, 1.4 and 2.0 M sucrose in 25 mM Tris · HCl, pH 7.4, and centrifuged 1.5 h at $65\,000 \times g$ in a SW-25 rotor. Individual membrane layers were removed and diluted with 25 mM Tris · HCl, pH 7.4, and pelleted at $105\,000 \times g$ for 1 h in a 50 Ti rotor. These pellets were resuspended in a small volume of homogenization medium, frozen and stored at −70 °C.

The supernatant from the $1080 \times g$ spin was centrifuged at $8000 \times g$ for 20 min in a Sorvall RC-2B refrigerated centrifuge, yielding a crude mitochondrial pellet. One portion of the post-mitochondrial supernatant was pelleted at $105\,000 \times g$ for 1 h to prepare a total microsomal fraction. The second portion was layered over 1.2 M sucrose and centrifuged for 3.5 h at $105\,000 \times g$ in a SW-50 Ti rotor to obtain the lipoprotein fraction of Ahmed and Williams-Ashman [3]. This latter centrifugation produced a pellet (M-2) and floating membrane layer (Fraction M-1) which were collected, diluted with 25 mM Tris · HCl, pH 7.4, and pelleted at $105\,000 \times g$. These fractions were resuspended in small quantities of homogenizing medium, frozen and stored at −70 °C.

(Na^+, K^+)-ATPase activity was the activity in the presence of $Mg^{2+} + Na^+ + K^+$ minus that in the presence of Mg^{2+} alone or $Mg^{2+} + Na^+$. Homogenates and membrane fractions were diluted with homogenizing medium so that 0.1 ml of enzyme source was added and in general less than 15% but not more than 30% of the substrate was hydrolyzed in the assay reactions. P_i released from ATP was linear for at least 15 min of incubation at 37 °C; the standard time adopted was 10 min. The reaction medium [3] contained 3 mM $MgCl_2$, 115 mM NaCl, 8 mM KCl, 3 mM disodium ATP, and 70 mM Tris · HCl, final pH 7.4 (at 25 °C); or 3 mM $MgCl_2$, 3 mM Tris · ATP, and 70 mM Tris · HCl in a total volume of 1 ml. The reaction was stopped by the addition of 2.0 ml of cold 10% (w/v) trichloroacetic acid containing 5 g acid-washed charcoal/100 ml [5]. The inorganic phosphate released was measured by the method of Fiske and SubbaRow [11]. Cytochrome oxidase was measured by the method of Cooperstein and Lazarow [12] and 5′-nucleotidase by the method of Morré [13].

DNA was measured in hot (70 °C) $HClO_4$ extracts using the method of Burton [14], with calf thymus DNA as a standard and RNA by the orcinol method [15] using yeast RNA as standard. Protein was determined in the $HClO_4$-extracted residues by the method of Lowry et al. [16] using bovine serum albumin as standard.

Membrane fractions were prepared for electron microscopy by fixation of the

pelleted fractions (105 000 $\times g$, 60 min) in glutaraldehyde, followed by OsO_4 and embedded in araldite-epon [17]. Membrane pellets were embedded such that cross-sections included areas from the top to the bottom of the pellet. Thin sections were stained with uranyl acetate and lead citrate [17].

RESULTS AND DISCUSSION

Prostatic plasma membranes were prepared from an initial low speed centrifugation (1080 $\times g$, 10 min) pellet by flotation on 1.6 M sucrose by centrifugation (65 000 $\times g$, 90 min) [10]. This fraction was shown to be enriched in (Na^+, K^+)-ATPase activity but the presence of cytochrome oxidase activity indicated some degree of mitochondrial contamination. It was further fractionated by centrifugation on a discontinuous sucrose gradient, yielding principal membrane layers at the 0.6-1.2 M and 1.2-1.4 M interfaces. The former was nearly free of cytochrome oxidase activity (0.2 % of the activity of the original homogenate) and contained 34.3 % of the (Na^+, K^+)-ATPase activity of the original homogenate (Table I). The membrane layer which floated on the 1.4 M sucrose (Fraction PM-2) contained 15.9 % of the (Na^+, K^+)-ATPase activity of the homogenate with a 3-fold increase in specific activity. This fraction, however, contained much of the mitochondrial contamination present before the discontinuous gradient step; 3.5 % of the cytochrome oxidase activity of the original homogenate was present in the PM-2 fraction. Mg^{2+}-stimulated ATPase and 5′-nucleotidase, also considered to be plasma membrane enzymes [18, 19], were not greatly enriched in either plasma membrane fraction: recoveries of activities were in the range of 2.4–6.5 % (Table I).

The specific activity of (Na^+, K^+)-ATPase present in the microsomal fraction was 2–3 times that in the homogenate and accounted for nearly 45 % of the total activity. Only low (Na^+, K^+)-ATPase activities were found in nuclear, mitochondrial and cytosol fractions. The microsomal fractions also contained 95 % of the Mg^{2+}-ATPase and 46 % of the 5′-nucleotidase activities. Nearly 35 % of the 5′-nucleotidase activity was recovered in the cytosol fraction. This enzyme may be one of the phosphatases present in seminal fluid secreted by the rat prostate and hence its presence in the soluble fraction may reflect contamination with prostatic fluid or disruption of secretory vesicles. The sum of the activities in the individual fractions accounted for 124 % of the (Na^+, K^+)-ATPase, 136 % of the Mg^{2+}-ATPase, and 121 % of the 5′-nucleotidase present in the original homogenate. Disruption and separation of subcellular fractions may have removed inhibitory molecules so that more than 100 % of the initial activity was recovered, calculated from the specific activities of the several fractions.

Fraction PM-1 contained 1.4 % (0.87 mg protein/g wet wt) and Fraction PM-2 accounted for 4.1 % (2.0 mg protein/g wet wt) of the protein in the initial homogenate. These values are similar to those reported for other tissues: 0.86 % recovery for salivary gland [20], 0.8 % for kidney [21], 1.7 % for lymphocytes [22] and a range for liver of 0.2–2.0 mg protein/g wet weight with some yields up to 8 mg protein/g [19]. The DNA content of the plasma membrane fraction was very small, at the limit of sensitivity of the method, but RNA (74 and 78 μg/mg protein for Fractions PM-1 and PM-2, respectively) was present. The RNA content may be due to the presence of ribosomes on membranes structurally attached to the plasma membrane [23] or to contam-

TABLE I

DISTRIBUTION OF (Na^+, K^+)-ATPase, Mg^{2+}-ATPase, 5′-NUCLEOTIDASE AND CYTOCHROME OXIDASE ACTIVITIES IN PLASMA MEMBRANE AND MICROSOMAL FRACTIONS OF THE RAT VENTRAL PROSTATE

	(Na^+, K^+)-ATPase		Mg^{2+}-ATPase		$Mg^{2+} + Na^+ + K^+ / Mg^{2+}$ ***	5′-Nucleotidase		Cytochrome oxidase	
	Spec. act.*	%**	Spec. act.*	%**		Spec. act.*	%**	Spec. act.†	%**
Homogenate	6.8†† (5.2–8.6)		35.8 (31.1–41.7)		1.19 (1.18–1.21)	2.4 (0.8–3.1)		0.196 (0.172–0.224)	
Fraction PM-1	36.6 (24.2–59.5)	34.3 (37.0–46.5)	73.3 (42.5–97.2)	6.5 (4.7–10.4)	1.68 (1.53–1.74)	3.8 (3.6–4.2)	4.8 (1.6–9.9)	0.015 (0–0.029)	0.2 (0–0.5)
Fraction PM-2	19.0 (18.2–20.4)	15.9 (13.7–22.3)	19.4 (17.5–21.0)	2.4 (1.9–3.0)	1.99 (1.83–2.07)	1.7 (1.4–2.0)	5.0 (2.6–7.3)	0.202 (0.187–0.220)	3.5 (3.2–4.1)
Microsomes	15.6 (12.0–17.6)	45.0 (22.7–68.5)	109.9 (84.9–125.7)	95.4 (86.2–116.1)	1.21 (1.18–1.24)	10.0 (5.0–14.3)	46.0 (25.9–57.3)	0.011 (0–0.18)	0.2 (0–0.4)

* μmol P_i/mg protein per h; $(Na^+ + K^+)$-ATPase activity was determined as the release of P_i from ATP in the presence of $Mg^{2+} + Na^+ + K^+$ minus that in the presence of Mg^{2+} alone or $Mg^{2+} + Na^+$.

** Percent of total homogenate activity recovered in fraction.

*** Ratio of ATPase activity in media with $Mg^{2+} + Na^+ + K^+ / Mg^{2+}$ alone.

† μmol reduced cytochrome *c* oxidized/mg protein per min.

†† Values are means of four preparations, range is given.

ination of the plasma membrane fractions with rough endoplasmic reticulum. However, RNA has been reported as a constituent of a number of plasma membrane preparations and has been suggested to be an integral part of plasma membranes [24, 25].

Electron micrographs of the PM-1 fraction revealed the morphology commonly found for plasma membranes; large membrane vesicles with occasional granular content and junctional complexes. Many of the membrane fragments were seen to be paired: relatively short tight junctions were often present but desmosomes were absent. In addition, no discernable microvilli were present in the micrographs. The micrographs also demonstrated the presence of occasional mitochondria and dense vesicular bodies which may be secretory vesicles similar to those described by Brandes [26] and Helminen and Ericsson [27]. Fraction PM-2 contained, in addition to plasma membrane vesicles, a greater population of mitochondria and secretory-like vesicles than Fraction PM-1.

The characteristics of prostatic plasma membrane (Na^+, K^+)-ATPase are similar to those reported for membranes of a prostatic microsomal fraction [3]: presumably such a fraction would contain a certain portion of the plasma membrane. The ratio of ATPase activity in the presence of $Mg^{2+}+Na^+ + K^+$ versus Mg^{2+} alone or $Mg^{2+}+Na^+$ was the same within each fraction and was 1.2 for homogenates (Table I). This ratio was 1.7 for Fraction PM-1 and 2.0 for Fraction PM-2, indicating the substantial ($Na^+ + K^+$)-dependent activity in these fractions. The ratio for microsomes, either total microsomes or the fraction floated on 1.2 M sucrose (Fraction M-1), was 1.2: the pellet of that centrifugation (Fraction M-2) had a ratio of 1.0. Although microsomes contained considerable (Na^+, K^+)-ATPase, the low ratio reflects the predominance of Mg^{2+}-ATPase in that fraction. The (Na^+, K^+)-ATPase was inhibited by ouabain or Ca^{2+}, results consistent with properties of (Na^+, K^+)-ATPase from other tissues [2]. 50 % inhibition of the (Na^+, K^+)-ATPase of Fraction PM-1 occurred at 0.33 mM ouabain and that of Fraction PM-2 at 0.15 mM (Fig. 1).

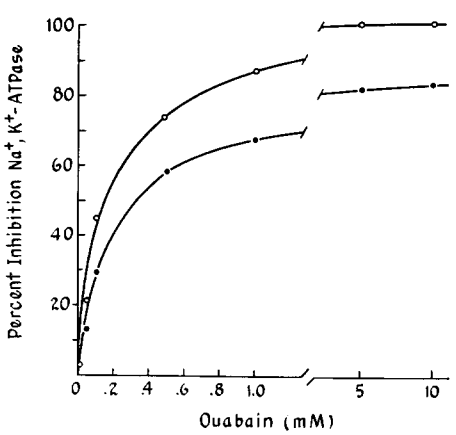

Fig. 1. The effect of varied ouabain concentrations on (Na^+, K^+)-ATPase activity. Enzyme activity was measured as described in Materials and Methods except that the membrane fractions were preincubated 10 min in the presence of buffer and cations and with or without ouabain: the reaction was started by the addition of ATP. The time of incubation was 10 min. The enzyme activity of Fraction PM-1 is plotted as ● — ● and that of Fraction PM-2 as ○ — ○.

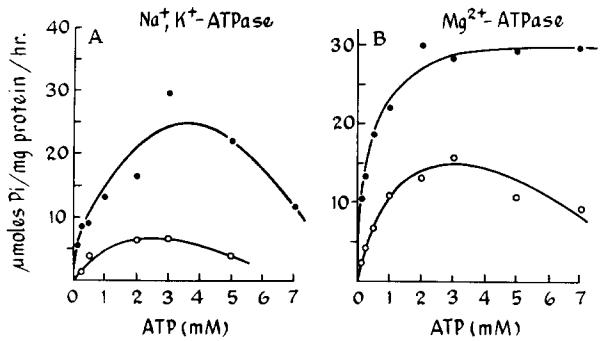

Fig. 2. (A) (Na^+, K^+)-ATPase and (B) Mg^{2+}-ATPase activities of plasma membranes as a function of ATP concentration. The enzymes were assayed as described in Materials and Methods, except that the ATP concentration was varied. The incubation time was 10 min and $MgCl_2$ was maintained at 3 mM. The enzyme activity for Fraction PM-1 is plotted as ●—● and that of Fraction PM-2 as ○—○.

(Na^+, K^+)-ATPase of Fraction PM-2 was completely inhibited at 10 mM ouabain whereas that of Fraction PM-1 was inhibited 83 % at this concentration. It was unexpected that the (Na^+, K^+)-ATPase of Fraction PM-2 showed a greater sensitivity to ouabain but this fraction also demonstrated a higher activity ratio than did Fraction PM-1 (Table I). However, the precise reason for this discrepancy is not clear: it may be related to differences in the rates of interaction of ouabain with these two membrane fractions and may not indicate the presence of a less ouabain-sensitive species of (Na^+, K^+)-ATPase.

Maximal activity for (Na^+, K^+)-ATPase and Mg^{2+}-ATPase was found at 3 mM ATP in both plasma membrane fractions (Fig. 2). However, the specific activity of both enzymes was much lower in Fraction PM-2. The $(Na^+ + K^+)$-dependent phosphatase activity was limited to ATP: no activity was evident with UTP, GTP or CTP as substrates. The Mg^{2+}-dependent hydrolysis with Fraction PM-1 and microsomes was in the order UTP > GTP > CTP > ATP (Table II), results consistent

TABLE II

Mg^{2+}-DEPENDENT PHOSPHATASE ACTIVITIES OF PLASMA MEMBRANE AND MICROSOMAL FRACTIONS OF RAT VENTRAL PROSTATE

The results are expressed as percent of activity compared to ATP as substrate. Mg^{2+}-dependent phosphatase activities were measured as in Materials and Methods for the ATPase assay except that the individual substrates (3 mM) were substituted for ATP. Incubation times were 5 min for UTP, GTP and CTP; 20 min for ADP and AMP. The release of P_i from these substrates was linear for these times of incubation.

Fraction	Percent of ATP activity				
	UTP	GTP	CTP	ADP	AMP
PM-1	154	140	124	40	3
PM-2	130	233	76	43	9
Microsomes	158	114	110	27	4

with those for prostatic microsomes [3]. Fraction PM-2 hydrolyzed GTP to a greater extent and CTP to a lesser extent than either microsomes or Fraction PM-1. This may be due do nucleotide phosphatase activity associated with mitochondria and/or secretory-like granules which were shown by electron microscopy to be the principal contaminating particles. In addition, there was no $(Na^+ + K^+)$-stimulated hydrolysis of ADP or AMP by these fractions, whereas the phosphatase activity toward ADP in the presence of Mg^{2+} alone (in the ATPase assay system) was about 40 % of the activity demonstrated toward ATP in homogenate, PM-1 and PM-2 fractions: this was 27 % in microsomes (Table II). The presence of ADPase activity has been demonstrated in plasma membranes of liver [28] and these data suggest a possible similar localization in prostate. However, we cannot rule out the possibility that some adenylate kinase may also be present in these preparations so that some ADP may be re-equilibrated to produce ATP+AMP [29] with the result that some of the P_i released may be derived from ATP. Likewise, through the adenylate kinase reaction it is possible that some P_i released in the ATPase assays may have been derived from the β-phosphate of the ATP originally added. However, it is unlikely that this factor should influence the specific activity of ATPase assays in the purified fractions, since adenylate kinase appears to be predominately localized in mitochondria with small quantities in the nucleus and cytosol [29], or in crude fractions (e.g. homogenate), since ATP substrate is already present in saturating amounts. Mg^{2+}-dependent hydrolysis of AMP was 3–4 % of the activity toward ATP in homogenate, Fraction PM-1, and microsomes, whereas it was 9 % in the PM-2 fraction. It was also noted above that this latter fraction differed from the former fractions with respect to the hydrolysis of GTP and CTP.

The plasma membrane fraction (Fraction PM-1) derived from rat ventral prostate was enriched in (Na^+, K^+)-ATPase activity but demonstrated low recoveries of Mg^{2+}-ATPase and 5′-nucleotidase activities. This suggests that these enzymes are present either in different portions of the plasma membrane and separated into different fractions by the procedures employed or are present in different subcellular organelles. The isolation of more than one plasma membrane fraction has been reported for other tissues. Nijjar and Pritchard [20] prepared three plasma membrane fractions from rat submandibular salivary gland: the fraction with highest specific activity for (Na^+, K^+)-ATPase demonstrated lower 5′-nucleotidase and Mg^{2+}-ATPase activity and, conversely, the fraction which possessed the highest 5′-nucleotidase and Mg^{2+}-ATPase activities had low (Na^+, K^+)-ATPase.

Evidence is accumulating which indicates a distinct topographical distribution of enzymes in the plasma membrane. The data for the prostatic plasma membrane preparations suggest that these membranes may have been derived primarily from the basal and lateral portions of the prostatic cells. Although gentle homogenization procedures were used in an attempt to minimize disruption of cells in the fibromuscular tissue, it is possible that a portion of the plasma membranes may have been contributed from this tissue component (in addition to the secretory epithelial cells) of the prostate gland. Brush border membranes of intestinal epithelial cells contain low (Na^+, K^+)-ATPase [30, 31] but high specific activities for sucrase, trehalase and leucyl-naphthylamidase [31], whereas lateral and basal plasma membranes are enriched in (Na^+, K^+)-ATPase and show only traces of sucrase [30]. In addition, lateral and basal intestinal epithelial cell surfaces, but not brush border, show binding

of [^{3}H]ouabain [32]. Differences in the distribution of enzymes in plasma membrane fractions from the kidney have also been reported. Purified renal brush borders possess alkaline phosphatase [33] and 5′-nucleotidase activities [34], whereas only low concentrations of these enzymes were found in renal plasma membrane preparations enriched in (Na^+, K^+)-ATPase and adenylate cyclase activities [21]. Heidrich et al. [35] have separated the plasma membranes of renal proximal tubule cells into two distinct fractions by preparative free flow electrophoresis. One fraction was highly enriched in (Na^+, K^+)-ATPase and the other in alkaline phosphatase activities: the former fraction demonstrated the presence of junctional complexes and the absence of microvilli upon examination by electron microscopy.

Should this correlation of specific distribution of plasma membrane marker enzymes apply, as implied, to the prostate gland, the preparative methods described here should be useful for studies of androgen-stimulated transport of substrate molecules, the interaction of prolactin with its receptor, the modulation of adenylate cyclase activity, and other functions which are considered properties of the plasma membranes of the serosal side of prostatic epithelial cells. This plasma membrane preparation should facilitate studies of possible androgen-(Na^+, K^+)-ATPase interactions in vitro [4–6]. However, studies from this laboratory [36] demonstrated that testosterone administration in vivo increases prostatic (Na^+, K^+)-ATPase activity 12 h but not 6 h after treatment. These data are inconsistent with a postulated mechanism of action by which androgen directly activates (Na^+, K^+)-ATPase [6].

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