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CALCIUM PUMP ACTIVITY OF THE RENAL PLASMA MEMBRANE AND RENAL MICROSOMES

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SUMMARY

ATP-dependent Ca^{2+} uptake distinct from that of the mitochondria is found in both plasma membrane and microsomal membranes of rat kidney. Activity attributed to these fractions is enhanced by ammonium oxalate and is apparently insensitive to NaN_3. In contrast, rat kidney mitochondrial Ca^{2+} uptake is blocked by NaN_3. The pH of optimal activity is significantly higher for the mitochondrial fraction. Microsomal membrane Ca^{2+} uptake differs from that of the plasma membrane. Microsomal membranes are four times as active as the plasma membrane at high (5 mM) ATP levels. Apparent K_m values for Mg^{2+}-ATP differ in the two preparations with a higher affinity for Mg^{2+}-ATP found in the plasma membrane. Ca^{2+} uptake activity of the plasma membrane preparation is readily inhibited by Na^+. Sucrose gradient density fractionation indicates that the observed microsomal membrane Ca^{2+} pump activity is associated with membrane vesicles derived from the endoplasmic reticulum. Ca^{2+} pump activity of both plasma membrane and microsomal fraction is depressed in the adrenalectomized rat. This activity is not restored by a single natriuretic dose of aldosterone.

INTRODUCTION

ATP-dependent Ca^{2+} sequestration has been extensively characterized in microsomal membrane vesicles derived from skeletal muscle sarcoplasmic reticulum [1–3]. Lower levels of this activity have been found in microsomal membranes isolated from mammalian smooth muscle [4, 5], brain [6–8], salivary gland [9] and platelets [10]. In addition this activity is found in ghost membranes of erythrocytes [11, 12] and in skeletal muscle plasma membrane [13, 14]. A plasma membrane Ca^{2+}-

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Abbreviation: SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid.

pumping mechanism is postulated to play a role in maintaining the electrochemical gradient for calcium between the external medium and cell interior [15].

Ca^{2+} has been shown to play a role in modifying permeability of renal tubule cells [16]. Palmer and Posey [17] have reported evidence for ATP-dependent Ca^{2+} binding in microsomal membranes prepared from rabbit kidney cortex [17]. We have recently isolated from rat kidney a subcellular fraction consisting of plasma membrane vesicles [18]. In the present study the existence of an ATP-dependent Ca^{2+}-pump activity in this plasma membrane preparation is demonstrated. This activity is also demonstrated in microsomal membrane vesicles isolated from the rat kidney. The Mg^{2+}-ATP-dependent Ca^{2+} uptake of the renal plasma membrane and microsomal membranes is characterized and compared with Mg^{2+}-ATP-dependent Ca^{2+} sequestering activity of renal mitochondria.

MATERIALS AND METHODS

The animals employed in this study were male rats of the Sprague–Dawley strain weighing approximately 250 g. Nucleotides employed in this study were obtained from Sigma chemical company $^{45}CaCl_2$ (8.0 mCi/mg calcium) was obtained from New England Nuclear Corp.

Rat kidneys were homogenized in an isotonic sucrose medium employing a Potter homogenizer with a Teflon pestle. Preparation of the subcellular fractions was carried out at 0–4 °C. For purposes of the present study EDTA was omitted from the sucrose media employed in homogenization and isolation of subcellular fractions. The plasma membrane fraction was prepared by a procedure described by Fitzpatrick et al. [18]. A "light" microsomal fraction was prepared according to the procedure of Landon and Norris [19]. Kidney mitochondria were isolated as previously described [20]. A succinate dehydrogenase assay according to the procedure of Pennington [21] was employed to monitor for mitochondrial contamination of the plasma membrane vesicles.

Calcium uptake was measured in the following medium: 100 mM KCl, 30 mM imidazole–histidine buffer, 5 mM ammonium oxalate, 5 mM NaN_3, 5 mM $MgCl_2$, 5 mM ATP (pH adjusted with Tris), 20 μM $CaCl_2$ and 0.07 μCi/ml $^{45}CaCl_2$ in a total volume of 3 ml. Plasma membranes were assayed at pH 6.8, microsomes at pH 6.6 and mitochondria at pH 7.4. These pH values were chosen for optimal activity. The assay was initiated at 37 °C by the addition of 300 μl of the subcellular fraction. The final protein concentrations were 0.2–0.4 mg/ml for the plasma membrane and microsomal fractions and 0.075–0.1 mg/ml for the mitochondrial fraction. Aliquots of 500 μl were removed for filtration through 0.45 μm membrane filters (Millipore Corporation) at 2, 10, 20 and 30 min. The filters were prepared with a wash of 0.25 M KCl (2 ml) followed by water (10 ml). Samples were filtered with the aid of a vacuum apparatus and were washed with 0.25 M sucrose (2 ml). The filtration in general followed the procedure described by Palmer and Posey [17]. The filters were dried and ^{45}Ca determined by liquid scintillation spectrophotometry in 2,5-diphenyloxazole (6 g/l) in toluene.

$(Mg^{2+}+Ca^{2+})$-stimulated ATPase activity of renal plasma membrane and renal microsomes was assayed with an incubation medium similar to that employed for measuring ATP dependent calcium uptake activity. However, no radioactive

calcium was necessary and the azide and oxalate were omitted. Calcium levels were varied from 0 to 100 μM. The reaction was terminated with 5% trichloroacetic acid and the liberated P_i was measured as previously described [19].

Protein content of the fractions was determined by the procedure of Sutherland et al. [22]. Nucleotides were identified on cellulose thin-layer chromatographic plates (Eastman Organic Chemicals) developed in *n*-butanol–acetone–$(NH_4)OH$–acetic acid–water (3.5 : 3.5 : 1.5 : 1.5 : 1, by vol.) according to the procedure described by Randerath [23]. The nucleotides were identified after incubation with the membrane fractions by spotting 5 μl of the 30-min incubation sample along with standard solution in the chromatographic system described above.

Density gradient studies were carried out in a continuous 20–50 percent sucrose gradient (w/v) containing 3 mM imidazole–histidine, pH 6.6. Approximately 2 mg of microsomal membrane protein were layered onto a 5 ml gradient that was then centrifuged at 130 000 $\times g$ for 16 h at 0 °C in a Beckman SW50L rotor. Gradient fractions were obtained by puncturing the bottom of the tube and collecting 0.7-ml samples. The following enzyme assays were carried out on the sucrose gradient fractions: (Na^+-K^+)-dependent ATPase [19] as a marker for plasma membrane and NADH oxidase [24] as a marker for endoplasmic reticulum.

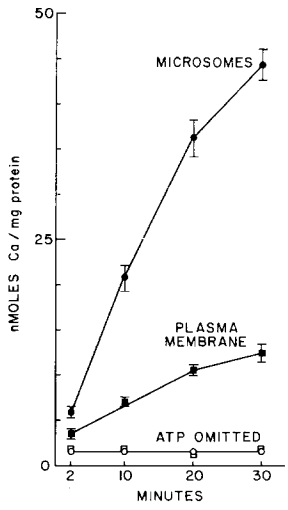
RESULTS

In Fig. 1, an Mg^{2+}-ATP dependent Ca^{2+} uptake by the plasma membrane preparation is demonstrated. In the absence of ATP approximately 2 nmoles Ca^{2+} per mg protein is bound to the plasma membrane fraction within the first 2 min of incubation. In the presence of 5 mM Mg^{2+}-ATP a continuous Ca^{2+} uptake is seen over a 30-min period. Microsomal activity is shown in the same figure and is seen to be about four times more active than that found in the plasma membrane.

Plasma membrane activity is characterized in more detail in Fig. 2. In this figure, non-ATP-dependent Ca^{2+} binding is subtracted. The Ca^{2+} uptake activity of the plasma membrane shown represents active uptake in presence of Mg^{2+}-ATP. In the absence of oxalate the Ca^{2+} uptake levels off between 10 and 20 min. In the presence of oxalate the uptake continues over the entire 30-min period. Stimulation of Ca^{2+} uptake by oxalate has previously been interpreted as evidence for translocation of calcium across the vesicle membrane in contrast to binding to external membrane sites [1]. Complexing of the oxalate anion with Ca^{2+} accumulated by the vesicles is thought to reduce the passive outflow so that energy dependent calcium accumulation is measured. It is possible to conceive the observed uptake of Ca^{2+} as a Ca^{2+} exchange between membrane and medium enhanced by the presence of Mg^{2+}-ATP. However, enhanced Ca^{2+} uptake activity observed in presence of oxalate makes this very unlikely.

When azide is omitted from the plasma membrane incubation there is some increase of Ca^{2+} uptake activity. This increase in approximately that to be expected from a two percent mitochondrial content of the plasma membrane preparation and is close to the three percent estimate of mitochondrial contamination based upon the succinate dehydrogenase assay.

Nucleotide triphosphates other than ATP do not support Ca^{2+} uptake (Fig. 3). Mg^{2+} is required for the demonstration of an ATP-dependent Ca^{2+} uptake. ADP



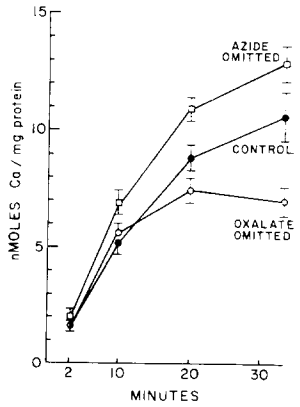
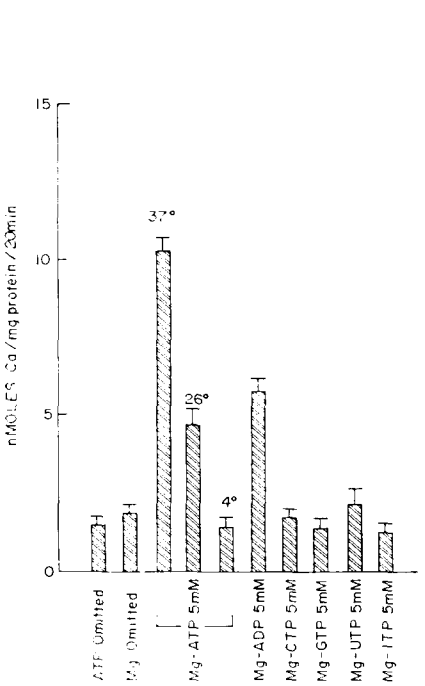


Fig. 1. Total Ca^{2+} uptake by rat renal plasma membrane and microsomal membrane fractions. Ca^{2+} uptake was measured in the following medium: KCl 100 mM, imidazole–histidine buffer 30 mM (plasma membrane pH 6.8, microsomes pH 6.6), ammonium oxalate 5 mM, NaN_3 5 mM, $MgCl_2$ 5 mM, $^{45}CaCl_2$ 20 μM (0.07 μCi/ml) with or without ATP (pH adjusted with Tris) 5 mM in a total volume of 3.0 ml at 37 °C. At zero time the membrane fraction was added (0.2–0.4 mg/ml, final concentration) to the prewarmed incubation medium. At each time point a 500-μl sample was removed, filtered and ^{45}Ca determined as described in the text. Each point represents the mean ± S.E. for the determination of Ca^{2+} uptake measured in six membrane preparations. With both membrane preparations the omission of ATP resulted in the binding of approximately 2 nmoles of Ca^{2+} per mg of membrane protein which was essentially complete by 2 min.

Fig. 2. Mg^{2+}-ATP dependent Ca^{2+} uptake by the rat renal plasma membrane. Ca^{2+} uptake was measured as described in Fig. 1. The binding of Ca^{2+} to the membrane in the absence of ATP was subtracted from the total Ca^{2+} uptake in the presence of Mg^{2+}-ATP. Each point represents the mean ± S.E. for six membrane preparations. After 20 min the presence of ammonium oxalate significantly ($p < 0.05$) increased the quantity of Ca^{2+} sequestered by the membrane fractions. The omission of NaN_3, an inhibitor of mitochondrial Ca^{2+} uptake, resulted in an increased uptake of Ca^{2+}. This difference is consistent with the small mitochondrial contamination of the plasma membrane preparations estimated by assay of succinate dehydrogenase activity.

will substitute for ATP with the plasma membrane preparation but it is considerably less effective (Fig. 3). The plasma membrane fraction was incubated with Mg^{2+}-ADP and the contents of the incubation analyzed by ascending thin-layer chromatography [23]. The plasma membrane preparation converts a portion of the nucleotide pool to ATP and AMP. We attribute the uptake of Ca^{2+} in the presence of Mg^{2+}-ADP to the myokinase activity found in the preparation. Active Ca^{2+} uptake by the plasma membrane preparation is temperature dependent. It is reduced at 26 °C and not detected at 4 °C (Fig. 3). The approximate Q_{10} for the Mg^{2+}-ATP-dependent reaction is three.

The microsomal membrane activity is characterized in more detail in Fig. 4. Non-ATP-dependent Ca^{2+} binding activity is subtracted so that the Ca^{2+} uptake activity of the microsomes that is shown in this figure is active uptake in presence of Mg^{2+}-ATP. Demonstration of Mg^{2+}-ATP-dependent Ca^{2+} uptake in the microsomal membranes is markedly stimulated by the presence of the oxalate anion. In



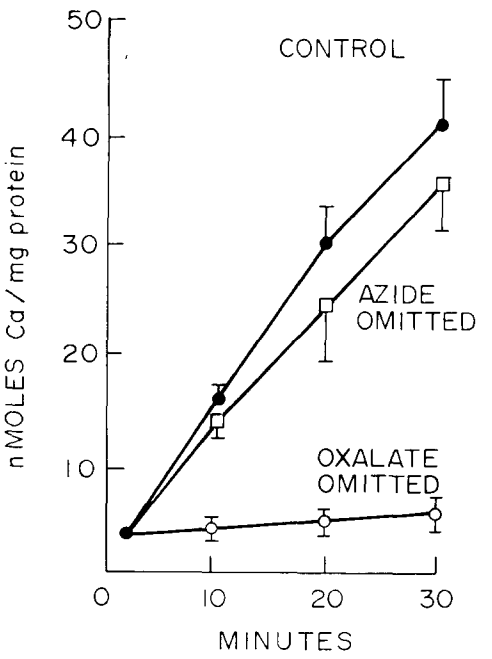


Fig. 3. Characteristics of total Ca^{2+} uptake by the rat renal plasma membrane preparation. Incubation medium for the control experiment is described in legend of Fig. 1. After 20 min of incubation at 37 °C a 500-μl sample was removed and ^{45}Ca was determined as described in the text. The substitution of other nucleotides for Mg^{2+}-ATP or the alteration of temper ature of incubation is indicated in the chart. Results are mean $\pm$ S.E. for six membrane preparations.

Fig. 4. Mg^{2+}-ATP dependent Ca^{2+} uptake by the rat renal microsomal membrane preparation. Ca^{2+} uptake was measured as described in Fig. 1. The binding of Ca^{2+} to the membrane in the absence of ATP was subtracted from the total Ca^{2+} uptake in the presence of Mg^{2+}-ATP. Each point represents the mean $\pm$ S.E. for six membrane preparations. At 10 min the presence of ammonium oxalate significantly increases quantities of Ca^{2+} sequestered by the membrane fraction.

contrast to findings with the plasma membrane, the omission of azide does not enhance the Ca^{2+} uptake. Other nucleotide triphosphates do not support active Ca^{2+} uptake (Fig. 5) and Mg^{2+} is required for the ATP dependent uptake. Limited activity is seen with Mg^{2+}-ADP and is attributed to the presence of some myokinase activity in the preparation. As in the plasma membrane preparation the Ca^{2+} uptake is markedly temperature dependent (Fig. 5). The approximate Q_{10} for the microsomal membrane Mg^{2+}-ATP dependent Ca^{2+} uptake is four.

Mg^{2+}-ATP dependent Ca^{2+} uptake activity versus the concentration of Mg^{2+}-ATP and Ca^{2+} is plotted for both microsomes (Fig. 6) and plasma membrane (Fig. 7). The calculated V is more than four times greater for the microsomal membranes. The calculated K_m for Mg^{2+}-ATP is 0.55 mM with the plasma membrane preparation and 3.12 with the renal microsomes. This apparent K_m for Mg^{2+}-ATP is significantly lower for the plasma membrane preparation. Estimates of the apparent K_m for Ca^{2+} gives values close to 20 μM in both preparations. The apparent K_m for

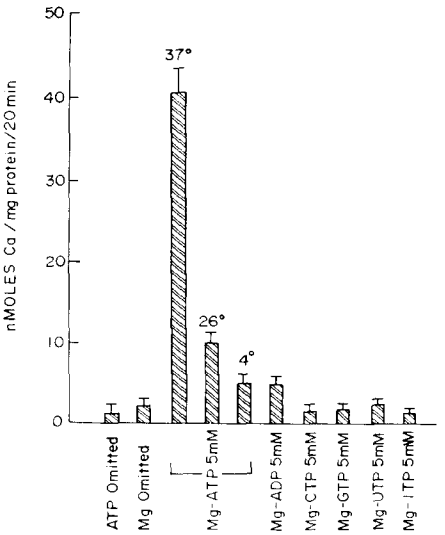


Fig. 5. Characteristics of total Ca^{2+} uptake by the rat microsomal membrane preparation. Incubation medium for the control experiment is described in legend of Fig. 1. After 20 min of incubation at 37 °C a 500-μl sample was removed and ^{45}Ca was determined as described in the text. The substitution of other nucleotides for Mg^{2+}-ATP or the alteration of temperature of incubation is indicated in the chart. Results are mean $\pm$S.E. for six membrane preparations.

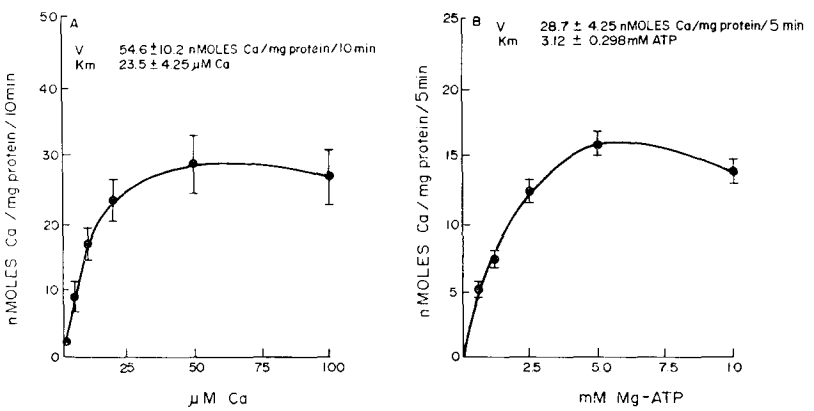


Fig. 6. Mg^{2+}-ATP-dependent Ca^{2+} uptake by the renal microsomal membrane preparation varying calcium and Mg^{2+}-ATP concentrations. Incubation medium essentially that described in legend of Fig. 1. After 10 min in (A) and 5 min in (B) a 500-μl sample was removed, filtered and ^{45}Ca determined as described in the text. In (A) the calculated V for Ca^{2+} uptake is 54.6$\pm$10 nmoles Ca^{2+} per mg protein in 10 min. The maximum observed velocity was 28.7 nmoles Ca^{2+} per mg protein in 10 min. The apparent K_m for Ca^{2+} is 23.5$\pm$4.25 μM. Each point in A represents mean$\pm$S.E. of five membrane preparations. In (B) the calculated V for Mg^{2+}-ATP is 28.7$\pm$2.15 nmoles Ca^{2+} per mg protein in 5 min. The apparent K_m for Mg^{2+}-ATP is 3.1$\pm$0.3 mM. Each point in B represents the mean$\pm$S.E. for eight experiments.

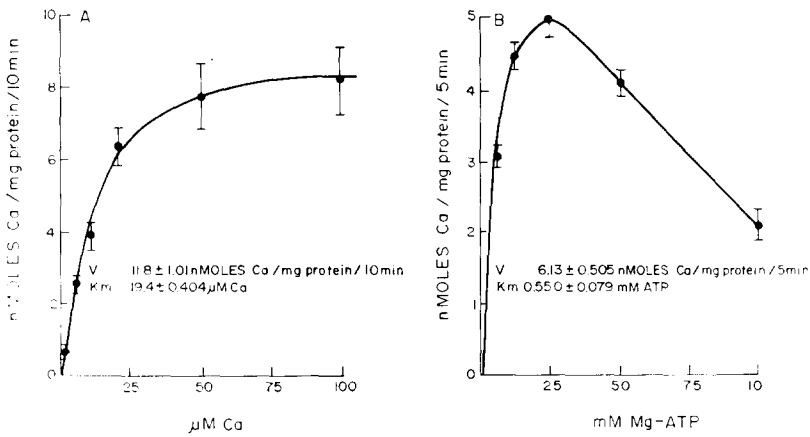


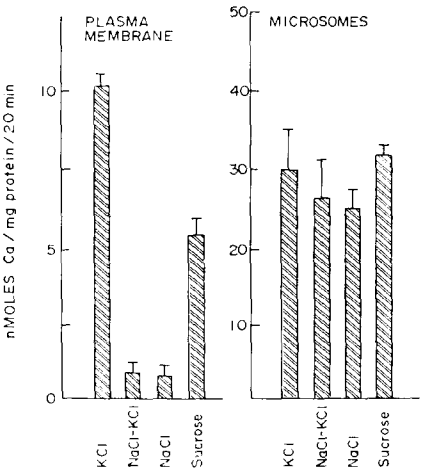
Fig. 7. Mg^{2+}-ATP dependent Ca^{2+} uptake by renal plasma membrane preparations varying Ca^{2+} and Mg^{2+}-ATP concentrations. The incubation medium is essentially that described in legend of Fig. 1. After 10 min in (A) and 5 min in (B) a 500-μl sample was removed, filtered and ^{45}Ca determined as described in the text. In (A) the calculated V for Ca^{2+} uptake is 11.8 ± 1 nmoles Ca^{2+} per mg protein in 10 min. The apparent K_m for calcium is 19.4 ± 0.4 μM. Each point represents the mean $\pm$S.E. of six experiments. In (B) the velocity of calcium uptake increased with increasing Mg^{2+}-ATP concentrations to 2.5 mM. At higher Mg^{2+}-ATP concentrations the Ca^{2+} uptake rapidly decreased. The apparent K_m for Mg^{2+}-ATP is 0.55 ± 0.08 mM which is significantly lower than that obtained for the microsomal membrane preparation.

Ca^{2+} in renal mitochondria is found in a similar set of measurements to be 126 ± 5 μM. Although in Fig. 1 with 5 mM Mg^{2+}-ATP the Ca^{2+} pump activity of microsomes is four times greater than that of the plasma membrane, this difference is much reduced at lower and perhaps more physiological levels of ATP.

An ATPase activity dependent on Mg^{2+} and stimulated by Ca^{2+} is associated with skeletal muscle microsomes and is large enough to potentially relate to the Ca^{2+}-sequestration system [25]. Ca^{2+}-stimulated Mg^{2+}-ATPase activity was measured in renal microsomes and plasma membrane with addition of 5, 20, 50 and 100 μM Ca^{2+}. Maximal ATPase was observed at 20 μM Ca^{2+} with renal plasma membrane and 100 μM Ca^{2+} with renal microsomes. The extra ATPase for 20 min averaged 600 nmoles phosphate per mg protein for the microsomes and 700 nmoles phosphate per mg protein for the plasma membrane.

The effect of replacing KCl in the medium with NaCl or sucrose is demonstrated in Fig. 8. Substitution by Na^+ virtually suppresses the Mg^{2+}-ATP dependent Ca^{2+} uptake of the plasma membrane preparation. Sucrose in iso-osmotic concentrations partially depresses the Mg^{2+}-ATP dependent uptake of the plasma membrane preparations. Sodium and sucrose have no demonstrable effect on Ca^{2+} uptake in the microsomal membrane preparation. When 1 mM EDTA is included in the sucrose medium employed in the initial homogenization of the renal tissue, the Mg^{2+}-ATP-dependent Ca^{2+} uptake activity of the microsomal and plasma membrane preparations is diminished about 40%. For this reason no EDTA was utilized in preparation of cell fractions.

Ca^{2+} uptake activity of kidney mitochondria has been previously studied [26, 27] but less commonly with the low levels of Ca^{2+} employed in the present



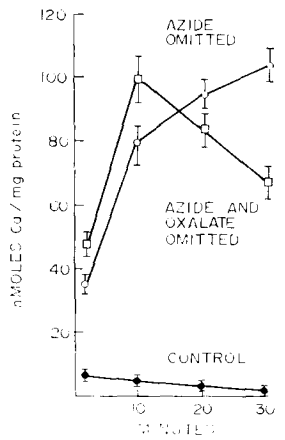


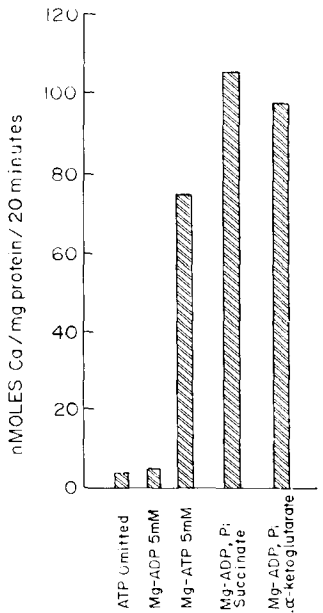
Fig. 8. The effect of the ionic composition of the assay medium on Mg^{2+}-ATP-dependent Ca^{2+} uptake by the plasma membrane and microsomal membrane preparations. Incubation medium for the control experiment (KCl) is described in legend of Fig. 1. After 20 min a 500-μl sample was withdrawn, filtered and ^{45}Ca determined as described in the text. Replacement of KCl 100 mM by NaCl 100 mM, sucrose 200 mM or by NaCl 50 mM and KCl 50 mM are indicated on the chart. Results are mean $\pm$ S.E. for six preparations.

Fig. 9. Mg^{2+}-ATP dependent Ca^{2+} uptake by rat renal mitochondria. Ca^{2+} uptake was measured in the following medium: KCl 100 mM, imidazole–histidine buffer (pH 7.4) 30 mM, ammonium oxalate 5 mM, NaN_3 5 mM, $MgCl_2$ 5 mM, ATP 5 mM, $^{45}CaCl_2$ 20 μM (0.07 μCi/ml) in a total volume of 3 ml at 37 °C. At zero time the mitochondrial suspension was added (0.075–0.1 mg/ml, final concentration) to the prewarmed incubation medium. At each time point a 500-μl sample was withdrawn, filtered and ^{45}Ca determined as described in the text. Binding of Ca^{2+} to the mitochondrial preparation in the absence of ATP was substracted from the total Ca^{2+} uptake in the presence of 5 mM Mg^{2+}-ATP. Each point represents the mean $\pm$ S.E. for Ca^{2+} uptake in six mitochondrial preparations. There is near total inhibition of active calcium uptake by 5 mM NaN_3. When oxalate is omitted the loss of Ca^{2+} after 10 min may represent a substantive increase in Ca^{2+} loss when oxalate is not available to remove free calcium from solution within the mitochondria.

experiments. Mg^{2+}-ATP-dependent Ca^{2+} uptake activity of kidney mitochondria is shown in Fig. 9. Mitochondrial Ca^{2+} uptake is completely inhibited by NaN_3, but in the absence of azide a potent Ca^{2+}-sequestration activity is demonstrated. Little or no oxalate effect is detected during the initial 10 min of mitochondrial Ca^{2+} uptake and there is no significant change when Na^+ replaces K^+. Mg^{2+}-ADP will not support Ca^{2+} uptake unless substrate and inorganic phosphate essential for mitochondrial systhesis of ATP are also present (Fig. 10).

Mg^{2+}-ATP dependent Ca^{2+} uptake in all three fractions was examined between pH 6.6 and 7.4. Maximum Ca^{2+} uptake by mitochondria is seen at pH 7.4. Maximum microsomal calcium uptake is seen at pH 6.6 and plasma membrane calcium uptake is optimal at pH 6.8 (Fig. 11).

The microsomal preparation is heterogeneous in its membrane composition. It consists of plasma membrane fragments and endoplasmic reticulum membrane fragments. Centrifugation of the microsomal fraction of a sucrose density gradient was carried out to partially separate plasma membrane from endoplasmic reticulum



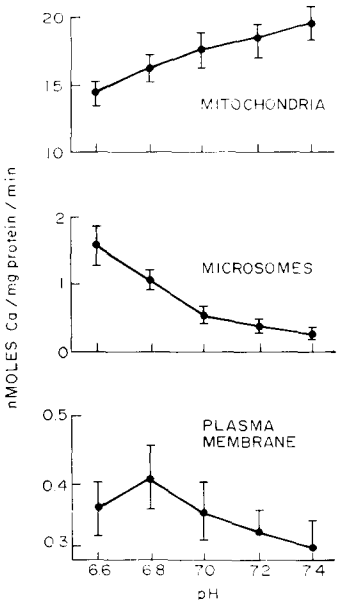


Fig. 10. Characteristics of total Ca^{2+} uptake by rat renal mitochondria. Incubation medium for these experiments omits azide and is described in the legend of Fig. 9. After 20 min of incubation at 37 °C a 500-μl sample was removed and ^{45}Ca was determined as described in the text. The effects of omitting Mg^{2+}-ADP or substituting other nucleotides are indicated on the chart. Each point represents the mean of three mitochondrial preparations.

Fig. 11. The effect of pH on Mg^{2+}-ATP stimulated Ca^{2+} uptake in rat renal mitochondria, plasma membranes and microsomal membranes. Determination of calcium uptake is described in the text with pH of the imidazole–histidine buffer noted in the figure. A sample was removed from the plasma membrane and microsomal membrane incubations at 10 min. The mitochondrial suspension was incubated for two minutes. Each point represents the mean $\pm$S.E. for six preparations of each membrane fraction.

membranes. Representative findings are depicted in Fig. 12. NADH oxidase is employed as a marker for endoplasmic reticulum and $(Na^+ + K^+)$-dependent ATPase for the plasma membrane. Ca^{2+} uptake appears to be associated with the endoplasmic reticulum marker enzyme in this preparation. Plasma membrane represents a minor component of this microsomal preparation and the weaker plasma membrane Ca^{2+} uptake activity would represent a very small part of the total activity observed in the microsomal preparation.

Mg^{2+}-ATP dependent Ca^{2+} pump activity has been examined in the plasma membrane preparation of rats adrenalectomized for 5–10 days. This activity is decreased by approximately 50% (Fig. 13). A single dose of aldosterone (50 μg per rat, 2 h before sacrifice of the animals) sufficient to restore Na^+ balance as measured by urinary electrolyte excretion [30] failed to increase Ca^{2+} pump activity in the test animals (Fig. 13). Findings were similar when the microsomal membranes were examined (Fig. 13).

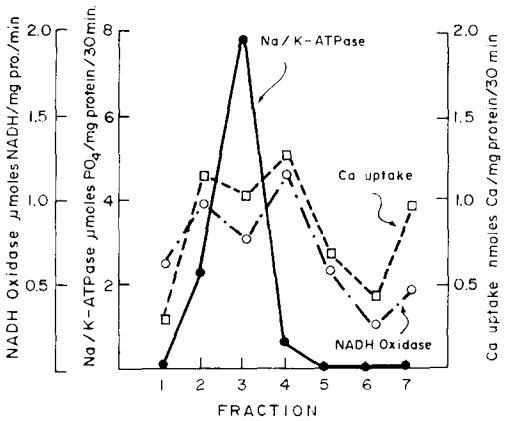


Fig. 12. Density gradient characterisation of the rat renal microsomal membrane preparation. Approximately 2 mg of the microsomal preparation was layered onto a 5-ml sucrose gradient (20–50 % (w/v)) containing 3 mM imidazole–histidine, pH 6.6. The gradient was centrifuged at 130 000 × *g* for 16 h in a Beckman SW50L rotor. Gradient fractions were obtained by puncturing the bottom of the tube and collecting seven 700-μl samples. Vesicles derived from the plasma membrane were characterized as those with ($Na^+ + K^+$)-dependent ATPase activity. Membrane vesicles from the endoplasmic reticulum were characterized as those associated with NADH oxidase activity. Ca^{2+} uptake activity appears to correlate with the distribution of NADH oxidase activity.

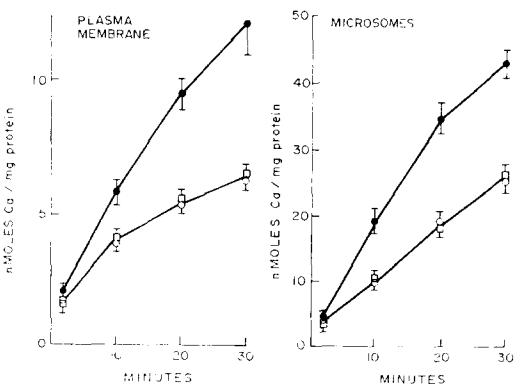


Fig. 13. Mg^{2+}-ATP dependent Ca^{2+} uptake activity in the plasma membrane and microsomal membrane preparations isolated from the adrenalectomized rat kidney. Rats were adrenalectomized 5–10 days before the assay and were maintained by replacement of drinking water with 1 % NaCl in tap water. A single dose of aldosterone (50 μg per rat, intraperitoneal) 2 h prior to isolation of the membranes failed to alter the decreased Ca^{2+} pump activity in the adrenalectomized rat. Each point represents the mean ± S.E. for eight plasma membrane experiments and 14 microsomal membrane experiments. The closed circle represents control rats. The open circle represents adrenalectomized rats and the square represents adrenalectomized rats that had received a single dose of aldosterone.

DISCUSSION

The identity of the plasma membrane fraction has been previously defined by enzyme markers [18]. The fraction consists of membrane vesicles when examined with the electron microscope. The fraction is enriched in marker enzyme activity for ($Na^+ + K^+$)-dependent ATPase and adenyl cyclase attributed to the plasma mem-

brane. The nonpenetrating fluorescent compound 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS) is found in this fraction when administered in vivo. The microsomal preparation contains glucose-6-phosphatase activity and a cytochrome b_5 spectrum in the Soret region when measured in the presence and absence of dithionite. These were absent in the plasma membrane vesicles indicating no significant microsomal endoplasmic reticulum contamination. Assay of succinate dehydrogenase activity in current plasma membrane preparations (EDTA omitted) indicates approximately 3% of the protein content of the fraction is of mitochondrial origin.

We have been able to demonstrate Mg^{2+}-ATP-dependent Ca^{2+}-binding activity in the plasma membrane and the microsomal membranes of rat kidney. This activity is clearly distinguished from that of mitochondria. Similar studies carried out in skeletal muscle demonstrate ATP-dependent Ca^{2+} binding in both the sarcolemma [13, 14] and membrane fragments of the sarcoplasmic reticulum [1–3]. The Ca^{2+} uptake activity of renal plasma membrane and microsomes is substantially different. Microsomal calcium uptake activity is greater than that of the plasma membrane. The apparent K_m for Mg^{2+}-ATP is higher in the microsomal membranes. At Mg^{2+}-ATP levels greater than 2.5 mM, the Ca^{2+} uptake by the plasma membrane fraction sharply decreases. This is in agreement with studies of Ca^{2+} uptake by fragments of erythrocyte membranes which are presumably derived from the plasma membrane of the cell [34]. Inhibition of Ca^{2+} uptake by high Mg^{2+}-ATP levels is not significant in the microsomal membranes.

The Ca^{2+} uptake activity of renal plasma membrane but not of the microsomal membranes is sensitive to the ionic composition of the assay system. Ca^{2+} uptake was significantly higher in 100 mM KCl than in iso-osmotic concentrations of NaCl or sucrose. In other isolated membrane systems effects of the ionic composition of the assay medium have been observed. Ca^{2+} uptake is somewhat decreased by sodium ions in heart microsomes [37] and in microsomes from crustacean peripheral nerve [38]. In contrast, either monovalent cation enhances the Ca^{2+}-stimulated, Mg^{2+}-dependent ATPase in the erythrocyte membrane [39, 40]. The differing characteristics of Ca^{2+} uptake by the microsomal and plasma membrane preparations outlined above along with the previous enzymatic caracterization [18] of the plasma membrane fraction, strongly suggest a Ca^{2+}-sequestering system distinct from and not attributable to microsomal contamination of the plasma membrane vesicles.

Plasma membrane and microsomal membrane Ca^{2+}-uptake activity, unlike that of the mitochondria, is insensitive to NaN_3. Furthermore, the apparent affinity for Ca^{2+} is much higher in both the plasma membrane and microsomal membrane fractions (K_m about 20 μM) than that observed in the mitochondrial fraction (K_m about 125 μM). NaCl had little effect on Ca^{2+} uptake activity of renal mitochondria in contrast to effects on the plasma membrane.

Ca^{2+} uptake activity of the microsomal membranes is seen in sucrose-density gradient studies to be associated with the endoplasmic reticulum. The small fragments of renal plasma membrane found in the total microsomal fraction would have low Ca^{2+} uptake activity and are not likely to contribute greatly to the Ca^{2+} pump activity observed in this fraction.

Nucleotide triphosphates other than ATP do not support active sequestration of calcium by either plasma membrane or microsomal membranes. This is in agreement with studies of isolated erythrocyte membrane fragments [34] and isolated

platelet membranes [10]. Although ATP appears to be the ultimate nucleotide triphosphate energy source for Ca^{2+} transport, resealed erythrocyte ghosts [35, 36] or erythrocyte membrane fragments with added cytosol [34] are able to transport Ca^{2+} with a variety of nucleotide triphosphates as the energy source.

Quantitatively Ca^{2+} uptake activity in kidney membranes is much less than that found in similar membranes obtained from skeletal muscle. For optimal activity one must work with fresh subcellular preparations. Oxalate is believed in skeletal muscle studies to enhance the Ca^{2+} transport by precipitating accumulated Ca^{2+} of the vesicle and thereby maintain a low concentration of free Ca^{2+} in the vesicle [31]. The locus of the calcium precipitate is not defined by the oxalate effect and may be either at the inner membrane or in the internal space of the vesicle. The demonstration of a stimulation of Ca^{2+} uptake by the oxalate anion in both the microsomal and plasma membrane vesicles prepared by our techniques would appear to be at variance with the observations of Palmer and Posey [17]. This, however, may not be the case. These authors studied Ca^{2+} uptake in fresh preparations of the microsomal fraction of rabbit renal cortex; a system which appears to have a substantially lower velocity of uptake than the rat kidney microsomes characterized in this report. When examined after 10 min incubation, the rabbit kidney microsomes had accumulated approximately 5 nmoles of Ca^{2+} per mg protein and no oxalate effect was apparent. In both the plasma membrane and microsomal membrane fractions from the rat kidney, we are unable to demonstrate a significant oxalate effect until greater than 10 nmoles of total Ca^{2+} per mg protein have been accumulated. This may suggest that the solubility product of calcium oxalate has not been exceeded in the rabbit kidney microsomal system.

An extra ATP splitting (($Mg^{2+}+Ca^{2+}$)-dependent ATPase) activity is expected with the demonstration of a Mg^{2+}-ATP dependent Ca^{2+} uptake activity. This ATPase activity has been demonstrated for skeletal muscle microsomes [25], erythrocyte ghosts [32] and aorta microsomes [4]. This ATPase activity was found in our study of the renal plasma membrane and the renal microsomal membranes.

There is need to demonstrate sufficient ATP hydrolysis to account for the effect of ATP on the Ca^{2+} uptake. The observed ATP hydrolysis is far in excess of Ca^{2+} uptake and the optimal concentration of Ca^{2+} differs for the uptake and ATP hydrolysis. This is not a unique characteristic of the renal Ca^{2+}-uptake systems. Similar results have been demonstrated in sacroplasmic reticulum vesicles from cardiac muscle (ref. 37, Fig. 2) and in vesicles from red blood cell membranes (ref. 41, Table III). Furthermore, Ca^{2+} uptake and Ca^{2+}-stimulated Mg^{2+}-ATPase activities in skeletal muscle sarcolemma have differing optimal pH and Ca^{2+} concentration [14, 42]. There are several possible explanations for the anomalous ATPase activity. The Ca^{2+} uptake may be only one of several activities coupled to Mg^{2+}-ATP hydrolysis by proteins of the cell membrane. Contractile proteins associated with the plasma membrane [43, 44] may well be expressed enzymatically as a Ca^{2+}-stimulated Mg^{2+}-ATPase. Another possibility is nonspecific uncoupling of the ATPase and membrane Ca^{2+} uptake resulting from vesicle damage associated with the preparative procedure. A third possibility is a mixed orientation of the isolated membrane vesicles. Steck et al. [45] have demonstrated that homogenization of human red blood cells results in a mixture of inside-out and right side-out plasma membrane fragments. Weiner and Lee [41] observed that inside-out properties have a much higher capacity for

Ca^{2+} sequestration. Ca^{2+}-stimulated Mg^{2+}-ATPase activity is not affected by the membrane vesicle orientation.

It has previously been shown that there is a reduced $(Na^+ + K^+)$-dependent ATPase activity in the rat kidney following adrenalectomy [28, 29] or following treatment with the aldosterone antagonist aldactone [29]. Dialysis of membrane particles after addition of 10 mM EDTA partially restores this activity in the adrenalectomized rat kidney membranes and completely restores this activity in the aldactone treated rat kidney membrane particles [29]. It was suggested that Ca^{2+} may play a role in the events described [29]. Evidence obtained in the present study would suggest that the inhibitory effect on the $(Na^+ + K^+)$-dependent ATPase activity seen with chronic adrenalectomy may be secondary to a depressed Ca^{2+} pump activity of the cellular membranes. This Ca^{2+} pump activity is decreased approximately 50% in the adrenalectomized rat.

The function of the Ca^{2+} pump is not known. Homeostasis of cytoplasmic calcium levels and regulation of membrane permeability are two possibilities. Borle [31] in a study of cultured renal cells, analyzed the desaturation curves of cellular calcium with a three compartment, open system model. Efflux of Ca^{2+} from extracellular space was rapid. Efflux from a cytoplasmic compartment was moderate. A considerably slower efflux was identified with energy dependent stores in subcellular compartments, presumably in mitochondria. The cytoplasmic compartment should be regulated in part by Ca^{2+}-pump activity of the plasma membrane and endoplasmic reticulum. The amount of this activity in the presence of low levels of calcium associated with plasma membrane and endoplasmic reticulum suggests that this cytoplasmic pump system plays an important role in the regulation of intracellular Ca^{2+}.

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