Biochimica et Biophysica Acta, 1022 (1990) 265–272
Elsevier

BBAMEM 74729

Stimulation of ATP-driven Ca^{2+} pump in the basal-lateral plasma membranes of kidney cortex during compensatory renal growth

Amela Hadžić *, Ivan Sabolić and Hrvoje Banfić

Department of Physiology, Faculty of Medicine, University of Zagreb, Zagreb (Yugoslavia)

(Received 11 September 1989)

Key words: ATPase, Ca^{2+}-; Sodium–calcium ion exchanger; Compensatory renal growth; Basal-lateral plasma membrane; (Kidney)

During compensatory renal growth $^{45}Ca^{2+}$ transport in basal-lateral plasma membrane vesicles isolated from the rat renal cortex have been investigated. Stimulation of Ca^{2+}-ATPase activity was observed, without an effect of compensatory renal growth on Na^+/Ca^{2+} exchanger activity and on passive Ca^{2+} permeability of the vesicles. Twelve hours following unilateral nephrectomy about 40% increase of Ca^{2+}-ATPase activity above control value was observed and this effect was present until the end of the experimental period (7 days). When kinetic parameters for Ca^{2+}-ATPase were studied in native membranes, an increase of V_{max} was observed, whereas the K_m for Ca^{2+} was similar in control vesicles and vesicles isolated from the remnant kidney. Depletion of endogenous calmodulin resulted in a decrease of V_{max} and an increase of K_m (Ca^{2+}), while its addition reversed these parameters and increased the Hill coefficient from about 1 to about 2. Once again, only a significant increase of V_{max} in vesicles isolated from the remnant kidney above the control value was observed. Finally, increase of Ca^{2+}-ATPase activity during compensatory renal growth could be abolished by actinomycin D, indicating that its stimulation is due to protein synthesis.

Introduction

After unilateral nephrectomy the remnant kidney undergoes compensatory growth during which there is an increase of kidney weight, RNA/DNA ratio, protein content, glomerular filtration rate and tubular reabsorption processes, leading to cell hypertrophy (for reviews, see Refs. 1 and 2). Although very little is known about the nature of stimulus which initiates compensatory renal growth, from our previous experiments it could be concluded that inositol lipid signaling is involved in its initiation [3–5], probably via mobilization of Ca^{2+} within the cells, as has been shown in many other experimental models [6,7]. In the renal cells the mechanisms for transport of the Ca^{2+} from the cells (Ca^{2+}-ATPase and Na^+/Ca^{2+} exchange) are localized in basal-lateral plasma membranes (BLPM), while the influx of Ca^{2+} into the cells is mediated particularly through brush-border plasma membranes [8,9]. Since it was observed that during compensatory renal growth the permeability for Ca^{2+} in brush-border membranes vesicles is not changed [10], we studied Ca^{2+} transport in BLPM only. Furthermore, we studied Ca^{2+} transport in the early phase of compensatory renal growth (first few hours following uninephrectomy) in which there is an increase in inositol lipid metabolism [3–5], stimulation of the Na^+/H^+ exchange in brush-border membranes [11], while the activity of renal Na^+/K^+-ATPase is unchanged [11,12].

Materials and Methods

Materials. Female albino Wistar rats from the departmental colony (3–4 months old) were used in experiments. $^{45}CaCl_2$ was from Amersham International. A23187 was purchased from Calbiochem. EGTA, ATP-Mg_2, vanadate, ouabain, calmodulin and valinomycin were from Sigma. Percoll was purchased from Pharmacia. Hexokinase was from Boehringer. Benzamil was from Merck, Sharp and Dome Res. Lab. Oligomycin and *N*-ethylmaleimide were from Serva. Cellulose acetate filters (0.6 μm pore size) were from Sartorius. All other chemicals were analytical grade.

Surgical procedures and isolation of BLPM. Either right-side unilateral nephrectomy or sham nephrectomy was performed by dorsolateral approach under diethyl ether anesthesia as described previously [3]. After differ-

Abbreviations: BLPM, basal-lateral plasma membranes; PTH, parathyroid hormone; IGF-I, insulin-like growth factor I.

Correspondence: H. Banfić, Zavod za fiziologiju, Medicinskog fakulteta, Šalata 3, P.O.B. 978, 41001 Zagreb, Yugoslavia.

ent periods of compensatory renal growth animals were killed by a blow on the neck and the kidneys were removed immediately. The BLPM were isolated from the kidney cortex exactly as described by Scalera et al. [13]. The gradient was unloaded from the bottom of the tube and 1 ml fractions were collected for study of the distribution pattern of marker enzymes on it. For Ca^{2+} transport studies fractions 17–21 were taken. To deplete the membranes of endogenous calmodulin, an aliquot of BLPM containing 4–5 mg protein was homogenized in 15 ml of 20 mM Hepes, 5 mM EDTA (pH 7.4). The procedure was further performed as described by Gmaj et al. [14]. Prepared membranes were stored in liquid nitrogen until use.

Measurement of $^{45}Ca^{2+}$ uptake. The $^{45}Ca^{2+}$ uptake was measured in a standard incubation medium which contained 100 mM KCl, 5 mM $MgCl_2$, 20 mM Tris-Hepes and 2 μCi of $^{45}Ca^{2+}$ (pH 7.0). In all experiments, with exception of kinetic studies, the 3.95 mM EGTA and 4.05 mM $CaCl_2$ were present in the incubation medium to give free Ca^{2+} concentration of 63 μM, which was calculated by procedure described in Ref. 15. Changes in the composition of the incubation medium are indicated in the legends to the figures and in the tables. The stop solution contained 100 mM KCl, 5 mM $MgCl_2$, 20 mM Tris-Hepes (pH 7.0) and 1 mM EGTA. Usually 50 μl of membrane suspension (protein concentration of 10 mg/ml) was added to 1 ml of incubation medium kept in a water bath at 37°C. At different time intervals a 100 μl sample was removed from the incubation medium and diluted into 1 ml of ice-cold stop solution and immediately filtered. The filter was then washed with 10 ml of ice-cold stop solution to remove extravesicular radioactivity. The radioactivity remaining on the filters was counted by standard liquid-scintillation techniques.

Measurement of $^{45}Ca^{2+}$ efflux. 50 μl of membrane suspension (protein concentration of 10 mg/ml) was preincubated for 45 min at 37°C in 700 μl of incubation medium which contained following substances (10 mM glucose, 5 mM ATP, 10 μM valinomycin and 2 mM ouabain) in addition to above mentioned medium for measurement of $^{45}Ca^{2+}$ uptake. At 45 min 10 μl of hexokinase (10 mg/ml) was added to convert ATP to ADP. At 48 min either KCl or NaCl, final concentration 30 mM, was added and at different time intervals a 100 μl sample was removed and processed as described above.

Other assays. Leucine arylamidase (EC 3.4.11.2) and alkaline phosphatase (EC 3.1.3.1) activities were determined colorimetrically at room temperature as described in the commercial kits (Merckotest Nos. 3359 and 3344). The activity of Na^+/K^+-ATPase (EC 3.6.1.3) was measured by the coupled optical assay at room temperature as described previously [16]. The orientation of the membrane vesicles was assessed by latency of Na^+/K^+-ATPase activity and its ouabain (2.4 mM) sensitivity before and after two cycles of freezing and thawing during incubation with 0.4 mg/ml deoxycholate [17].

Statistical evaluation. All results are shown as means ± S.E. For statistical analysis the Student's *t*-test for independent data and the regression analysis were used.

Results

The distribution pattern of the marker enzymes

Na^+/K^+-ATPase for the basal-lateral membranes and alkaline phosphatase and leucine arylamidase for the brush-border membranes on the Percoll gradient are shown in Fig. 1. The position of the peak activity for Na^+/K^+-ATPase is the same for the kidney membranes isolated either from sham operated or uninephrecto-

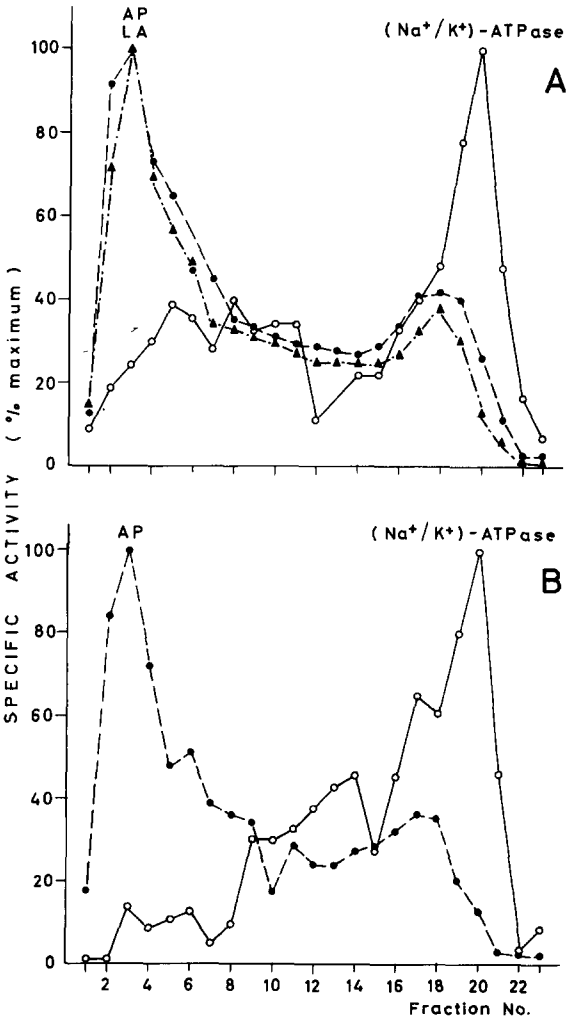

Fig. 1. Distribution of plasma membrane marker enzymes on Percoll gradients. (A) Plasma membranes from sham operated animals. (B) Plasma membranes from remnant kidney 2 days after unilateral nephrectomy. ○, Na^+/K^+-ATPase; ●, alkaline phosphatase (AP); ▲, leucine arylamidase (LA). Each point represents a single measurement from the representative experiment repeated three times.

TABLE I

The orientation of the basal-lateral membrane vesicles isolated from kidney cortex 2 days after either uninephrectomy (Uni) or sham operation (Sham)

The data are expressed as percent of maximal Na^+/K^+-ATPase activity and are from three independent experiments.

	Sham	Uni
Unassignable	62.3 ± 3.7	58.6 ± 4.1
Right-side-out vesicles	27.1 ± 2.6	28.2 ± 3.3
Inside-out vesicles	12.1 ± 1.2	13.2 ± 2.1

mized animals. The enrichment factor for Na^+/K^+-ATPase in the 'sham operated' BLPM was (10.5 ± 1.2, $n = 18$) while in the 'uninephrectomized' BLPM it was (10.8 ± 1.3, $n = 13$), which is very similar to the results from previous reports [17–20]. The enrichment factors for the brush-border membranes marker enzymes were in both experimental conditions about 1.

The orientation of the membrane vesicles is shown in the Table I. There was no significant difference in 'sidedness' of BLPM vesicles isolated either from sham operated or uninephrectomized animals. These observations are also in agreement with those of Scoble et al. [17]. Despite the heterogeneity of BLPM vesicles, only tight inside out vesicles take Ca^{2+} in the presence of extravesicular ATP, which allowed us to study an ATP-dependent Ca^{2+} transport using the heterogeneously oriented vesicles.

Inhibition of ATP-dependent Ca^{2+} uptake by different compounds was examined by measuring the uptake of $^{45}Ca^{2+}$ in the presence of ATP after 5 min of incubation (Table II). There was no statistical difference in the action of different inhibitors between BLPM vesicles isolated from kidneys obtained after

TABLE II

Effects of different inhibitors on ATP-dependent Ca^{2+} uptake in basal-lateral membrane vesicles isolated from kidney cortex 2 days after uninephrectomy (Uni) or sham operation (Sham)

The data are from three independent experiments and are expressed as percent of Ca^{2+} uptake in untreated vesicles, which was 4.61 ± 0.19 nmol of Ca^{2+}/5 min per mg of protein in control (Sham) vesicles, while in the vesicles isolated from remnant kidney (Uni) it was 7.02 ± 0.22 ($P < 0.01$). The vesicles were pretreated with different inhibitors for 15 min at 4 °C and inhibitor was also present during incubation period (5 min). * $P < 0.01$ compared with the corresponding control.

Addition	Sham	Uni
Control	100.0 ± 4.1	100.0 ± 3.1
Oligomycin (1 μg/ml)	88.6 ± 9.9	85.2 ± 5.4
Ouabain (2 mM)	88.0 ± 2.6	86.0 ± 6.8
Benzamil (1 mM)	104.7 ± 5.7	96.2 ± 3.0
Vanadate (1 μM)	53.1 ± 4.3 *	42.4 ± 5.7 *
N-Ethylmaleimide (1 mM)	34.2 ± 1.1 *	36.1 ± 9.6 *

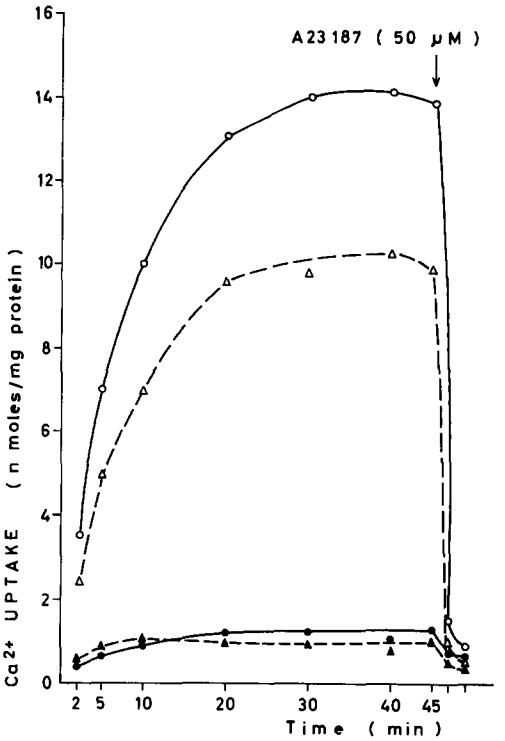

Fig. 2. Influx of Ca^{2+} into basal-lateral plasma membrane vesicles: effects of ATP (5 mM), uninephrectomy and A23187 (50 μM). Open symbols represent Ca^{2+} uptake in the presence of ATP. Membrane vesicles isolated from kidney cortex 2 days after either uninephrectomy (●, −ATP; ○, +ATP) or sham operation (▲, −ATP; △, +ATP). Each point represent a single measurement from the representative experiment repeated three times.

sham operation or uninephrectomy. The ATP dependent Ca^{2+} uptake in BLPM vesicles was practically insensitive to oligomycin (inhibitor of mitochondrial Ca^{2+} uptake), ouabain (inhibitor of Na^+/K^+-ATPase) and benzamil (inhibitor of Na^+ binding site in Na^+/Ca^{2+} exchanger). On the other hand the uptake was sensitive to vanadate (inhibitor of P type ATPases) and *N*-ethylmaleimide (alkylator of free sulfhydryl groups) and both of these compounds, in similar concentrations, are well known inhibitors of plasma membrane Ca^{2+} pump in the BLPM vesicles isolated from the kidney cortex [20,21].

Above mentioned results (Fig. 1, Tables I and II) demonstrate together that the renal BLPM isolated from uninephrectomized rats do not differ from those isolated from sham operated animals in their distribution pattern on Percoll gradient, marker enzyme enrichment factor, orientation of the membrane vesicles and sensitivity of Ca^{2+} uptake to different inhibitors and could therefore be used for study of Ca^{2+} transport across the BLPM during compensatory renal growth.

The influx of $^{45}Ca^{2+}$ into BLPM vesicles in the presence and absence of ATP is shown in Fig. 2. In the absence of ATP Ca^{2+} influx was much smaller than in the presence of ATP and there was no statistical dif-

ference in the influx between BLPM vesicles isolated from sham operated or uninephrectomized animals (results not shown). When Ca^{2+} ionophore A23187 was added at the end of the incubation period the radioactivity accumulated in the vesicles could only be partially released and the amount of Ca^{2+} left in the vesicles did not differ between BLPM vesicles isolated from sham operated or uninephrectomized animals (results not shown). These findings seem to indicate that Ca^{2+} influx into BLPM vesicles represents not only the permeability of the vesicles for Ca^{2+}, but also shows binding of the Ca^{2+} in the vesicle interior, as has been demonstrated previously [18,22]. Moreover, both of these processes are not influenced by uninephrectomy, while in the brush-border membranes isolated from the remnant kidney a decreased Ca^{2+} binding for the vesicles due to a decreased membrane content of acidic phospholipids was demonstrated, while permeability of the vesicles for Ca^{2+} was not changed [10].

As can be seen in Fig. 2, the addition of ATP to the BLPM vesicles provoked a rapid Ca^{2+} uptake, which was significantly increased in the BLPM vesicles isolated from the remnant kidney (see legend to Fig. 3). The radioactivity accumulated in the vesicles could be almost completely released after the addition of A23187 and the residual radioactivity left in the vesicles was the same as that in the experiments were ATP was omitted (results not shown).

The kinetic properties of ATP-dependent Ca^{2+} uptake in native, calmodulin depleted as well as in calmodulin depleted vesicles in which exogenous calmodulin was added are shown in the Table III. The kinetic properties of Ca^{2+}-ATPase in native BLPM vesicles isolated from sham operated animals are similar to those observed by Gmaj et al. [18]. Depletion of endogenous calmodulin from the membranes resulted in an increase of K_m (Ca^{2+}) and a decrease of V_{max}, while upon the addition of exogenous calmodulin a typical reversal in these kinetic parameters appeared together with an increase of the Hill coefficient from about 1 to about 2 (results not shown), as has already been described [8,14,21,23]. It is generally assumed that Ca^{2+}-calmodulin complex activates the Ca^{2+}-ATPase via a mixed-type affinity-velocity effect, i.e., it decreases the K_m (Ca^{2+}) and increases the V_{max} simultaneously [23–25]. When the kinetic properties of Ca^{2+}-ATPase in native BLPM vesicles isolated from uninephrectomized animals were studied, a significant increase of the V_{max} in comparison with the vesicles isolated from sham operated animals was observed without change in the K_m (Ca^{2+}). Depletion of endogenous calmodulin resulted in an increase of K_m (Ca^{2+}) and a decrease of V_{max} and there was no statistical difference in these parameters between 'sham-operated' and 'uninephrectomized' BLPM vesicles, although V_{max} was slightly higher in 'uninephrectomized' BLPM vesicles. Addition of exogenous calmodulin resulted in a decrease of K_m (Ca^{2+}), an increase of V_{max} and an increase of the Hill coefficient from about 1 to about 2 (results not shown), while once again the only significant difference in comparison with the vesicles isolated from sham operated animals was observed in the V_{max}. The observation that in calmodulin-depleted BLPM vesicles isolated from uninephrectomized animals there is no significant increase of V_{max} when compared with control BLPM might be explained, that in such a circumstance the Ca^{2+}-ATPase is present mostly in a low-affinity, low-velocity state, so that the influence of compensatory renal growth on the enzyme activity could not be demonstrated.

Time course of increase of wet kidney weight, Na^+/K^+-ATPase activity and ATP-dependent Ca^{2+} uptake during various periods following unilateral nephrectomy are shown in the Fig. 3. It is well known that during compensatory renal growth the wet weight and the Na^+/K^+-ATPase activity of the remnant kidney increase [2,12], this was also observed in the present study and could be further extended for the Ca^{2+}-ATPase activity. Moreover, when kinetic parameters for Na^+/K^+-ATPase were studied an increase of V_{max} was

TABLE III

Kinetics of ATP-dependent Ca^{2+} uptake by basal-lateral membrane vesicle isolated from kidney cortex 2 days after either uninephrectomy (Uni) or sham operation (Sham)

The free Ca^{2+} concentration was varied from 0.13 μM to 16.39 μM by adding different concentrations of EGTA and $CaCl_2$ as suggested in Ref. 15 and uptake was measured with five different concentrations of free Ca^{2+}. The uptake was measured during 2 min of incubation period in the presence and absence of 5 mM ATP and the uptake in the absence of ATP was subtracted from the uptake in the presence of ATP. The linearity of double-reciprocal plot from each single experiment was analyzed by regression analysis and in all experiments r^2 was > 0.952. Each data are from three independent experiments. * $P < 0.01$ compared with the corresponding control.

	K_m (Ca^{2+}) (μM)		V_{max} (nmol/10 min per mg protein)	
	Sham	Uni	Sham	Uni
Native membranes	0.53 ± 0.06	0.49 ± 0.04	9.86 ± 0.09	13.35 ± 0.54 *
Membranes extracted with hypotonic EGTA				
− calmodulin	0.85 ± 0.05	0.87 ± 0.05	6.88 ± 0.16	7.33 ± 0.20
+ calmodulin (3 μg/ml)	0.28 ± 0.02	0.29 ± 0.02	11.77 ± 0.07	16.29 ± 0.55 *

observed [26] as we could demonstrate for Ca^{2+}-ATPase. The same authors also noticed [26] that during compensatory renal growth there is an increase of surface area of the basal-lateral plasma membranes and an increase of ouabain-binding sites in the proximal tubular cells, which suggests new membrane protein synthesis. Since we observed previously [11] that treatment of animals with actinomycin D (an inhibitor of protein synthesis) abolished the stimulation of Na^+/K^+-ATPase activity during compensatory renal growth, we used actinomycin D to test the possibility that an increase of Ca^{2+}-ATPase activity following unilateral nephrectomy is due to increased protein synthesis. As shown in Table IV treatment of animals with actinomycin D abolished the increase of ATP-dependent Ca^{2+} uptake in BLPM

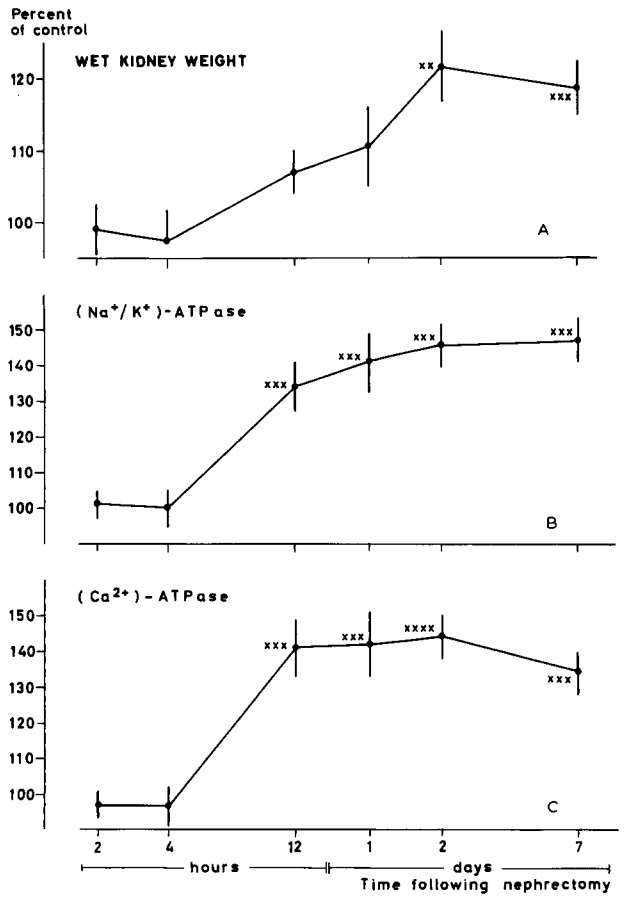

Fig. 3. Time course of increase in wet kidney weight, Na^+/K^+-ATPase activity and ATP-dependent Ca^{2+} uptake during compensatory renal growth. For each time point wet kidney weight was estimated on four sham operated and four uninephrectomized kidneys, while for other parameters duplicate determinations in three independent experiments were performed, also from both sham operated and hypertrophied kidney membranes. Two days after sham operation the activity of Na^+/K^+-ATPase (nmol/min per mg of protein) was 382.5 ± 39.0 and ATP-dependent Ca^{2+} uptake was (nmol/5 min per mg of protein) 4.73 ± 0.16 (A), whereas in hypertrophied kidney membranes the activity of Na^+/K^+-ATPase was 558.3 ± 36.2 (B) and ATP-dependent Ca^{2+} uptake was 6.86 ± 0.12 (C). The results are expressed as percentages of the corresponding controls (means ± S.E.). * $P < 0.01$, ** $P < 0.001$ compared with the corresponding control.

TABLE IV

Effect of actinomycin D on ATP-dependent Ca^{2+} uptake in basal-lateral membrane vesicles isolated from kidney cortex 2 days after either uninephrectomy (Uni) or sham operation (Sham)

Actinomycin D was injected intraperitoneally at a dose of 0.1 mg/kg body mass 30 min before uninephrectomy and thereafter every 12 h for the next 2 days. Ca^{2+} uptake was measured during 5 min of incubation period. The data are from three independent experiments. * $P < 0.01$ compared with corresponding control.

	Ca^{2+} uptake (nmol/5 min per mg protein)	
	Sham	Uni
Control	4.61 ± 0.19	7.02 ± 0.22 *
Actinomycin D	3.58 ± 0.38	3.51 ± 0.23

vesicles isolated from kidneys undergoing compensatory growth. This together with the unchanged K_m (Ca^{2+}) indicates that the adaptation in the maximum rate of transport achieved in the BLPM vesicles for Ca^{2+}-ATPase is probably due to an increase in the number of functional pump sites as has been demonstrated for Na^+/K^+-ATPase [26].

The effect of sodium on the efflux of Ca^{2+} from BLPM vesicles is shown in Fig. 4. The efflux of Ca^{2+} in the presence of a Na^+ gradient (extravesicular Na^+ concentration higher than intravesicular Na^+ concentration) is significantly higher ($P < 0.01$) over that observed in the presence of a K^+ gradient. The significance was calculated on the basis of the difference between regression coefficients of straight lines derived from regression analysis for each ion (Na^+ or K^+) during initial two minutes following the addition of the ion. When the kidney BLPM vesicles were loaded passively with $^{45}Ca^{2+}$ the addition of Na^+ to extravesicular space results in the dissipation of Ca^{2+} from the vesicles, which is linear for not more than about 10 s [17,18,22,27]. In our experiments we used not only passive Ca^{2+} permeability of BLPM to load the vesicles, but by addition of ATP we used also Ca^{2+}-ATPase. Since in the presence of ATP the uptake of Ca^{2+} is increased about 10-fold above that in its absence (Fig. 2) the observation that dissipation of Ca^{2+} is linear for 2 min is not surprising. Because the addition of benzamil, an inhibitor of the Na^+ binding site in the Na^+/Ca^{2+} exchanger [27,28], abolished the increased dissipation of Ca^{2+} when Na^+ was added to extravesicular space, without influencing the passive calcium permeability of the vesicles in the presence of K^+ gradient (Fig. 4) and activity of Ca^{2+}-ATPase (Table II), we believe that we could demonstrate the Na^+/Ca^{2+} exchanger activity in BLPM vesicles. When BLPM vesicles were isolated from the remnant kidney cortex 2 days after unilateral nephrectomy there was no difference in Na^+/Ca^{2+} activity when compared with the vesicles isolated from sham operated animals and an identical result was

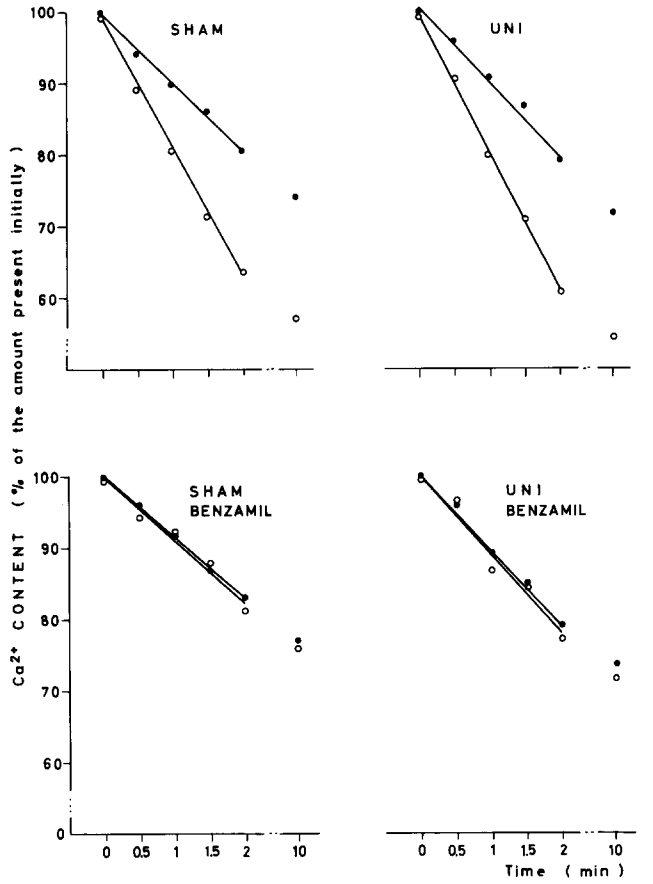

Fig. 4. Effect of sodium on the efflux of Ca^{2+} from basal-lateral plasma membrane vesicles isolated from kidney cortex 2 days after either uninephrectomy (Uni) or sham operation (Sham). ○, the efflux from BLPM vesicles in the presence of a sodium gradient; ●, the efflux from BLPM vesicles in the presence of a potassium gradient. Each point represents a single measurement from the representative experiment which was repeated three times. The results are expressed as percentage of the amount of Ca^{2+} found in the vesicles before starting efflux, which amounted to 7.92 ± 0.57 nmol/mg of protein in Sham vesicles and to 10.71 ± 0.35 nmol/mg of protein in Uni vesicles. Benzamil in the final concentration of 1 mM was added together with hexokinase and further details are as in Materials and Methods. The linearity during the first two minutes of reaction was analyzed by regression analysis and in all experiments r^2 was > 0.961.

obtained when vesicles were isolated 2 h following unilateral nephrectomy (results not shown). Finally, dissipation of Ca^{2+} from the vesicles in the presence of K^+ gradient, which represents passive Ca^{2+} permeability of the vesicles, was also not influenced by unilateral nephrectomy and the same result was obtained when the passive influx of $^{45}Ca^{2+}$ into the vesicles was studied (Fig. 2).

Discussion

Recently we observed that plasma collected 15–180 min following unilateral nephrectomy possesses renotropic activity and stimulates phospholipase C in the intact renal cortical slices with a consequent cellular increase in second messengers, inositol 1,4,5-trisphosphate, inositol 1,3,4,5-tetrakisphosphate and diacylglycerol [3,4] which are involved, in some cells, in initiation of their growth by stimulation of Na^+/H^+ exchanger [7]. Furthermore, short-term and long-term stimulation of Na^+/H^+ exchange in cortical brush-border membranes during compensatory renal growth were observed. In the first few hours following unilateral nephrectomy (short-term stimulation) the Na^+/H^+ exchange is stimulated most likely via phosphorylation of exchanger molecules by protein kinase C activation, while in the later period (long-term stimulation) its stimulation depends on protein synthesis and an increase of number of exchanger molecules in the brush-border membranes of the remnant kidney [11].

Since inositol 1,4,5-trisphosphate mobilizes Ca^{2+} from endoplasmic reticulum, while inositol 1,3,4,5-tetrakisphosphate helps former in sustained intracellular increase of Ca^{2+} concentration [29], the present investigation was undertaken to study short-term and long-term influences of compensatory renal growth on Ca^{2+} transport in BLPM vesicles, because in the kidney cells the mechanisms for transport of Ca^{2+} from the cells are localized in BLPM [8,9], while it has been already demonstrated that influx of Ca^{2+} into brush border membrane vesicles is not changed during compensatory renal growth [10]. It should be pointed out, that investigation of Ca^{2+} transport in isolated vesicles may not completely reflect the Ca^{2+} movements in the intact kidney cells during compensatory renal growth. Unfortunately due to the high autofluorescence rate of the plasma we were not capable to measure an increase, if any, of cytosolic free Ca^{2+} concentration using fura-2 in cultured renal epithelial cells using plasma taken from animals undergoing compensatory renal growth (Bonventre and Banfić, unpublished observation).

The present results demonstrate that among the mechanisms for Ca^{2+} transport (Na^+/Ca^{2+} exchanger and Ca^{2+}-ATPase) in isolated BLPM vesicles from the remnant kidney only the latter is stimulated (increase of V_{max}) during compensatory renal growth. Furthermore these results suggest that the general pattern of activation of the Ca^{2+}-ATPase after addition of exogenous calmodulin is not different in BLPM vesicles isolated from uninephrectomized from that in sham operated animals (an increase of V_{max}, a decrease of K_m for Ca^{2+} and an increase of Hill coefficient) and that such pattern of activation obey a typical behavior characteristic for deactivated enzyme depleted of calmodulin [8,14,18,21,23–25].

It is not astonishing that during compensatory renal growth Na^+/Ca^{2+} exchanger is not stimulated, since recently it has been observed that Na^+/Ca^{2+} exchange system is located exclusively in the distal tubule [30], which does not undergo adaptation (hypertrophy) in an extensive manner during compensatory renal growth

[1,2]. Since we use whole kidney cortex for the preparation of the vesicles our demonstration of Na^+/Ca^{2+} exchange in them could be the result of different vesicles populations originated from proximal and distal tubule. Furthermore, if any of Na^+/Ca^{2+} exchangers are present in the proximal tubular cells probably they are close to or at equilibrium and do not catalyze Ca^{2+} transport either out or into the cells [9].

Giving the above mentioned reservation that investigation of Ca^{2+} transport in isolated vesicles may not reflect the Ca^{2+} movements through the intact membrane, the observation that stimulation of Ca^{2+}-ATPase activity could only be noticed 12 or more hours following unilateral nephrectomy and could be attenuated by actinomycin D, suggests that there are no short-term mechanisms of Ca^{2+}-ATPase activation as has been demonstrated for stimulation of Na^+/H^+ exchanger activity in brush-border membrane vesicles [11].

It is tempting to speculate about the stimulus for activation of Ca^{2+}-ATPase. The increase in glomerular filtration rate which occurs in the remnant kidney already 2 hours following unilateral nephrectomy [31] could result in increased 'need' for reabsorption of Ca^{2+} in the proximal tubules, which might be a stimulus for Ca^{2+}-ATPase activation. The observation that the reabsorption of Ca^{2+} in proximal tubules occurs mostly via paracellular pathways and that only about 20% of filtered Ca^{2+} is reabsorbed actively via transtubular transport [32] together with the observation that the increase in glomerular filtration rate due to ureteroperitoneostomy will not stimulate Na^+/K^+-ATPase [33], which behaves similarly as Ca^{2+}-ATPase during compensatory renal growth (Refs. 12, 26 and this study), suggests that stimulation of Ca^{2+}-ATPase in the remnant kidney is probably not due to an increase of glomerular filtration rate. On the other hand, the increased Ca^{2+}-ATPase activity could be a late consequence of unknown humoral factor(s) from plasma which activates phospholipase C [3], or might be a result of action of some hormone or growth factor, which stimulates Ca^{2+}-ATPase activity in native renal BLPM and also influences renal growth, such as PTH or IGF-I [34,35]. It has been demonstrated that maintenance of animals on a low calcium diet (which stimulates PTH production) or administration of exogenous PTH to animals leads to renal hypertrophy [36]. Since PTH stimulates inositol trisphosphate and diacylglycerol production in renal tubular cells [37], as we could also observe for plasma obtained from uninephrectomized animals [3], the PTH might be a candidate which stimulates Ca^{2+}-ATPase during compensatory renal growth, although in isolated BLPM vesicles PTH also stimulates the passive Ca^{2+} permeability and Na^+/Ca^{2+} exchange [17,22,27], which we could not observe during compensatory renal growth. IGF-I could also stimulate Ca^{2+}-ATPase activity in BLPM [35] and it has been demonstrated that IGF-I, infused subcutaneously into hypophysectomized rats, stimulates renal hypertrophy [38], while its infusion in healthy man increases the glomerular filtration rate [39]. Moreover, its mRNA as well as its local concentration increases in the remnant kidney [40,41], while its addition to intact renal cortical slices stimulates phospholipid synthesis [42], which was also observed during compensatory renal growth [43]. These observations leave the possibility that the increase of Ca^{2+}-ATPase activity during compensatory renal growth might result from an IGF-I action.

To summarize, present investigation demonstrates that in the isolated BLPM vesicles from the remnant kidney there is a long-term stimulation of Ca^{2+}-ATPase activity, probably due to an increase in the number of functional pump sites in the BLPM, without effect of compensatory renal growth on either the Na^+/Ca^{2+} exchanger activity or the passive Ca^{2+} permeability of the vesicles, but further experiments will be necessary to clarify the 'trigger' and exact mechanism of Ca^{2+}-ATPase activation.

References

1 Fine, L.G. (1986) Kidney Int. 29, 619–634.
2 Wesson, L.G. (1989) Nephron 51, 149–184.
3 Banfić, H. and Kukolja, S. (1988) Biochem. J. 255, 671–676.
4 Banfić, H. (1989) Biochim. Biophys. Acta 1002, 273–276.
5 Banfić, H. and Gatalica, Z. (1989) Biochem. J. 260, 365–369.
6 Berridge, M.J. and Irvine, R.F. (1984) Nature (London) 312, 315–321.
7 Berridge, M.J. (1987) Biochim. Biophys. Acta 907, 33–45.
8 Van Os, C.H. (1987) Biochim. Biophys. Acta 906, 195–222.
9 Gmaj, P. and Murer, H. (1988) Miner. Electrolyte Metab. 14, 22–30.
10 Ferrari, C. and Esbrit, C. (1988) Miner. Electrolyte Metab. 14, 297–301.
11 Salihagić, A., Mačković, M., Banfić, H. and Sabolić, I. (1988) Pflügers Arch. 413, 190–196.
12 Katz, A.I. and Epstein, F.H. (1967) Yale J. Biol. Med. 40, 222–230.
13 Scalera, V., Huang, Y.K., Hildman, B. and Murer, H. (1981) Membr. Biochem. 4, 49–61.
14 Gmaj, P., Zurini, M., Murer, H. and Carafoli, E. (1983) Eur. J. Biochem. 136, 71–76.
15 Bulos, B.A. and Sacktor, B. (1979) Anal. Biochem. 95, 62–72.
16 Berner, W. and Kinne, R. (1976) Pflügers Arch. 361, 269–278.
17 Scoble, J.E., Mills, S. and Hruska, K.A. (1985) J. Clin. Invest. 75, 106–1105.
18 Gmaj, P., Murer, H. and Kinne, R. (1979) Biochem. J. 178, 549–557.
19 Tsukamoto, Y., Suki, W.N., Liang, C.T. and Sacktor, B. (1985) J. Biol. Chem. 261, 2718–2724.
20 Tsukamoto, Y., Tamura, T. and Muramo, F. (1988) Biochim. Biophys. Acta 945, 281–290.
21 Gmaj, P., Murer, H. and Carafoli, E. (1982) FEBS Lett. 144, 226–230.
22 Jayakumar, A., Cheng, L., Liang, C.T. and Sactor, B. (1984) J. Biol. Chem. 259, 10827–10833.
23 Rasmunssen, H. and Waisman, D.M. (1983) Rev. Physiol. Biochem. Pharmacol. 95, 111–148.
24 Cox, J.A., Comte, M., Malnoe, A., Burger, D. and Stein, E. (1984) in Metal Ions in Biology, Vol. 17. Calcium and its Role in

Biological systems (Siegel, H., ed.), pp. 215–273, Marcel Dekker, New York.
25 Gmaj, P., Bianchi, G. and Murer, H. (1988) Biochim. Biophys. Acta 941, 187–197.
26 Salehmoghaddam, S., Bradley, T., Mikhail, N., Badie-Dezfooly, B., Nord, E.P., Trizna, W., Kheyfets, R. and Fine, L.G. (1985) Lab. Invest. 53, 443–452.
27 Scoble, J.E., Cragoe, Jr., E.D. and Hruska, K.A. (1988) Biochim. Biophys. Acta 944, 233–241.
28 Kaczorowski, G.J., Slaughter, R.S., King, V.F. and Garcia, M.L. (1989) Biochim. Biophys. Acta 988, 287–302.
29 Irvine, R.F., Moor, R.M., Pollock, W.K., Smith, P.M. and Wreggett, K.A. (1988) Phil. Trans. Roy. Soc. B. 320, 281–298.
30 Ramachandran, C. and Brunette, M.G. (1989) Biochem. J. 257, 259–264.
31 Allison, N.E.M., Liphman, E.M., Lassiter, W.E. and Gottschalk, C.W. (1973) Kidney Int. 3, 354–363.
32 Ullrich, K.J., Rumrich, G. and Klöss, S. (1976) Pflügers Arch. 364, 223–228.
33 Fanestil, D.D. (1968) Nature (London) 218, 176–177.
34 Itoh, K., Morimoto, S., Shiraisi, T., Taniguchi, K., Onishi, T., Kumahara, Y. (1988) Biochem. Biophys. Res. Commun. 150, 263–270.
35 Lévy, J., Gavin, R., Morimoto, S., Hammerman, M.R. and Avioli, L.V. (1986) Endocrinology 119, 2405–2411.
36 Jobin, J., Taylor, C.M., Caverzasio, J. and Bonjouir, J.P. (1984) Am. J. Physiol. 246, F685–F690.
37 Hruska, K.A., Urowskowitz, D., Esbrit, P., Civitelli, R. and Westbrook, S. (1987) J. Clin. Invest. 79, 230–239.
38 Guler, H.P., Zapf, J., Scheiwiller, S. and Froesh, E.R. (1988) Proc. Natl. Acad. Sci. USA 85, 4889–4892.
39 Guler, H.P., Schmid, C., Zapf, J. and Froesch, E.R. (1989) Proc. Natl. Acad. Sci. USA 86, 2868–2872.
40 Fagin, J.A. and Melmed, S. (1987) Endocrinology 120, 718–724.
41 Stiles, A.D., Sosenko, I.R.S., D'Ercole, J.A. and Smith, B.T. (1985) Endocrinology 117, 2397–2401.
42 Crljen, V. and Banfić, H. (1989) Biochim. Biophys. Acta 1012, 24–28.
43 Banfić, H., Irvine, R.F. and Pokrajac, N. (1985) Biochim. Biophys. Acta 833, 223–228.