Inositol lipid signalling occurs in brush-border membranes during initiation of compensatory renal growth in the rat

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Using highly specific mass assays, concentrations of inositol lipids and 1,2-diacylglycerol (DAG) were determined in plasma membranes isolated from rat kidney cortex. Significantly higher concentrations of inositol lipids were determined in brush-border (BBM) than in basal-lateral (BLM) plasma membranes, although DAG concentrations were similar in both. After unilateral nephrectomy, a decrease in $\text{PtdIns}(4,5)\text{P}_2$ and $\text{PtdIns}4\text{P}$, with a concomitant increase in DAG and translocation of protein kinase C (PKC), were observed in BBM but not in BLM isolated from the remaining kidney. On the other hand, stimulation of renal cortical slices with insulin-like growth factor II (IGF-II) or phenylephrine caused similar effects in BLM but not in BBM.

Stimulation of phospholipase C activity with translocation of PKC only to BBM in one kidney was also induced by occlusion of blood flow through the contralateral kidney for 15 min. At 30 min after the occlusion was removed and reflow established, DAG concentration and the amount of PKC in BBM returned to control values. These results suggest that an early signal after unilateral nephrectomy is transmitted to cells through BBM and can be switched on and off by blood occlusion and reflow through the contralateral kidney, while hormonal signals caused by IGF-II and phenylephrine are transmitted to cells through BLM.

INTRODUCTION

After unilateral nephrectomy, a variety of functional, biochemical and morphological changes develop in the remaining kidney, compensating to a great extent the loss of one kidney (Fine, 1986; Wesson, 1989). Although the biochemical nature and the origin of the stimulus (or stimuli) that triggers compensatory renal growth is still unclear, recent studies performed *in vitro* demonstrate that plasma from uninephrectomized rats produces complex signalling in renal cortical slices based on phospholipase C activation (Banfić, 1990; Banfić et al., 1992). Furthermore, the increase in activity of the Na^+/H^+ exchange in the brush-border plasma membranes (BBM) could already be observed 15 min after unilateral nephrectomy (Salihađić et al., 1988), whereas no increase in activity of other transport processes in kidney plasma membranes, e.g. $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$, $\text{Ca}^{2+}\text{-ATPase}$, $\text{Na}^+/\text{Ca}^{2+}$ exchanger, $\text{Na}^+/\text{HCO}_3^-$ cotransporter and $\text{H}^+\text{-ATPase}$, could be seen in such a short time period (Hadžić et al., 1990; Jurčević et al., 1992; Salihađić et al., 1988). This suggests a close important relationship between inositol lipid signalling pathways and activation of Na^+/H^+ exchanger during initiation of compensatory renal growth, as has also been suggested for cell growth in other experimental models (Berridge and Irvine, 1989; Moole-naar et al., 1983).

The following study was performed to determine whether: (1) inositol lipid signalling occurs after unilateral nephrectomy *in vivo*; (2) the signalling is localized in BBM or basal-lateral plasma membranes (BLM), or both; (3) it could be switched on and off by blood occlusion and reflow through contralateral kidney, as demonstrated for Na^+/H^+ exchanger activity (Salihađić et al., 1988).

MATERIALS AND METHODS

Materials

Percoll, DEAE-Sepharose and Blue-Sepharose were from Pharmacia. Phenylephrine, insulin growth factor II (IGF-II) and anti-rabbit IgG conjugated to horseradish peroxidase were from Sigma. Pyruvate kinase, lactate dehydrogenase and phosphoenolpyruvate were from Boehringer. Silica gel plates were from Merck. $[\text{32P}]\text{ATP}$ (3000 Ci/mmol) was from either Amersham or ICN. All other chemicals used were of p.a. grade purchased commercially.

Animals and treatment

Studies were performed on Wistar rats of both sexes aged 3–4 months. The rats were bred at the Department of Physiology, School of Medicine, University of Zagreb, Croatia. During the experiments, the rats were offered pelleted food (Sljeme, Zagreb, Croatia) and tap water *ad libitum*.

Unilateral nephrectomy was performed under light ether narcosis by the dorsolateral approach as described previously (Banfić et al., 1985). If not stated otherwise, the right kidney was removed. Control animals were sham-operated.

Renal cortical slices from intact animals were prepared after the animals had been killed by a blow on the neck. The kidneys were removed, washed with ice-cold isotonic saline and stripped of capsule and fat. Sagittal cortical slices weighing 30–50 mg were cut from each side of the kidney with a Stadie–Riggs microtome. Two slices were placed into a 25 ml Erlenmeyer flask with 2 ml of Krebs–Ringer bicarbonate medium (pH 7.4) con-

taining 10 mM glucose. The flasks were gassed with 95 % O_2 /5 % CO_2 , capped and incubated at 37 °C in a shaking water-bath for various time periods (Kukolja et al., 1986).

Preparation of BBM and BLM

Rat renal cortical BBM were isolated by the Mg/EGTA precipitation method (Biber et al., 1981). Compared with the homogenate, the isolated membranes were enriched in brush-border marker enzymes [leucine arylamidase (13.7 ± 0.5 , $n = 25$, homogenate = 1) and alkaline phosphatase (7.8 ± 0.3 , $n = 12$)] similar to published values (Salihagić et al., 1988), and the enrichment factor for $(Na^+ + K^+)$ -ATPase activity, a marker for contraluminal membranes, was 0.7 ± 0.1 ($n = 10$). Unilateral nephrectomy did not affect any of the enrichment factors for the above-mentioned marker enzymes (results not shown).

The BLM were isolated from the kidney cortex using a Percoll gradient method as described by Scalera et al. (1981). The enrichment factor for $(Na^+ + K^+)$ -ATPase was 10.6 ± 0.4 ($n = 19$), whereas the enrichment factors for the BBM marker enzymes (leucine arylamidase and alkaline phosphatase) were 1.2 ± 0.2 ($n = 17$) and 1.1 ± 0.1 ($n = 9$) respectively, similar to those published previously (Hadžić et al., 1990). Unilateral nephrectomy did not affect any of the enrichment factors for the above-mentioned marker enzymes (results not shown).

Mass assay of lipid extraction and quantification

Diacylglycerol (DAG) from the plasma membranes was extracted using 0.75 ml of chloroform/methanol (1:2, v/v). When inositol lipids were to be measured, membranes were extracted using 0.75 ml of chloroform/methanol/1 M HCl (80:160:1, by vol.). Further extraction was performed as described by Folch et al. (1957). Measurements of inositol lipid concentration were performed as described below. For measurement of DAG concentration, lipid extract was dissolved in 0.5 ml of chloroform, loaded on a silicic acid column (0.5 ml made in a Pasteur pipette), eluted with 1 ml of chloroform and dried again; DAG was then determined as described below.

DAG

DAG kinase purification was achieved in a single step from rat brain by using a DEAE-Sepharose column as described by Divecha and Irvine (1990). DAG concentration was determined as follows. The dried lipid was dissolved in 20 μ l of CHAPS (9.2 mg/ml) and sonicated at room temperature for 15 s. After the addition of 80 μ l of buffer (50 mM Tris/acetate, 80 mM KCl, 10 mM magnesium acetate, 2 mM EGTA, pH 7.4), the reaction was started by the addition of 20 μ l of DAG kinase followed by 80 μ l of buffer containing 5 μ M ATP and 1 μ Ci of [32 P]ATP. After 1 h at room temperature, the reaction was stopped by the addition of 750 μ l of chloroform/methanol/HCl (80:160:1, by vol.). Phosphatidic acid was extracted as described by Folch et al. (1957) and chromatographed on oxalate (1 %)-sprayed t.l.c. plates using the following solvent system: chloroform/methanol/conc. ammonia/water (45:35:2:8, by vol.). After autoradiography, the spots corresponding to phosphatidic acid were scraped off and their 32 P content was determined by liquid-scintillation counting. The DAG content in each sample was adjusted to between 5 and 50 pmol, as the sensitivity of the mass assay is highest in this range (Divecha et al., 1991).

PtdIns P_2

To the dried PtdIns P_2 was added 150 μ l of monomethylamine reagent, prepared as described by Clarke and Dawson (1981),

and the sample was incubated at 53 °C for 60 min. The sample was dried and 100 μ l of 50 mM sodium periodate was added. After 30 min at room temperature, 20 μ l of ethylene glycol (10 %, v/v) was added to stop the reaction. After 15 min, 50 μ l of 1 % (v/v) 1,1'-dimethylhydrazine/formic acid (pH 4) was added, and the mixture was allowed to stand for a further 1 h. The pH of the sample was adjusted to 7 with 1 M Tris/HCl and the sample was evaporated to dryness. Preparation of binding protein and the procedure for the binding assay for Ins(1,4,5) P_3 was exactly as described by Palmer and Wakelam (1990). The PtdIns P_2 content in each sample was adjusted to between 0.5 and 5 pmol, as the sensitivity of the mass assay is highest in this range (Divecha et al., 1991).

PtdInsP

This assay was performed using a highly purified bovine brain PtdInsP kinase as described by Divecha et al. (1991). Mass measurement was performed as follows. Standard quantities of PtdInsP or lipid extract from cell membranes were dried by vacuum centrifugation and resuspended in 100 μ l of buffer (50 mM Tris, 10 mM $MgSO_4$, 80 mM KCl, 2 mM EGTA, pH 7.4), followed by sonication in a water-bath. The reaction was started by the addition of 100 μ l of the above-mentioned buffer containing 20 μ l of enzyme, 10 μ M ATP and 1 μ Ci of [32 P]ATP (3000 Ci/mmol). After 1 h at room temperature, the reaction was terminated by the addition of 750 μ l of chloroform/methanol/conc. HCl (80:160:1, by vol), and the lipids were extracted as in Folch et al. (1957). After separation of lipids by t.l.c. (as for the DAG assay), PtdIns P_2 spots were scraped off, after identification by autoradiography, and quantified by liquid-scintillation counting. The PtdInsP content in each sample was adjusted to between 2 and 16 pmol, as the sensitivity of the mass assay is highest in this range (Divecha et al., 1991).

PtdIns

PtdIns mass measurement was performed using a coupled assay employing a mixture of PtdIns-specific phospholipase C and the DAG kinase as described previously (Divecha et al., 1991). The dried lipid was dissolved by the addition of 50 μ l of buffer (80 mM Tris/acetate, 10 mM magnesium acetate, 2 mM EGTA, 10 mM KCl, 70 mM NaCl, pH 7.4) and sonicated for 15 s. Phosphatidylethanolamine (50 nmol) in 80 μ l of the above buffer was then added. The reaction was started by the addition of 20 μ l of enzyme mixture followed by 50 μ l of buffer containing 5 μ M ATP and 1 μ Ci [32 P]ATP. The reaction was continued for 1 h at room temperature and phosphatidic acid was extracted and quantified as for the DAG assay (above). The PtdIns content in each sample was adjusted to between 5 and 50 pmol, as the sensitivity of the mass assay is highest in this range (Divecha et al., 1991).

Western blotting with anti-PKC antibody

Cell membranes were resuspended in 300 μ l of final buffer [10 mM Tris/HCl (pH 8.0), 5 mM $MgCl_2$, 0.32 M sucrose, 5 mM 2-mercaptoethanol and 1 mM EGTA]. Protein (50 μ g) was subjected to SDS/PAGE (10 % gel). Transfer to nitrocellulose was achieved using 10 mM Na_2CO_3 /3 mM $NaHCO_3$ buffer (pH 9.9) for 1.5 h. The blot was blocked in buffer (20 mM Tris/HCl (pH 7.4), 0.5 M NaCl, 5 % BSA, 0.2 % Tween 20), followed by incubation of primary antibody (1:1000) overnight at 4 °C in the above buffer. After incubation with anti-rabbit IgG conjugated to horseradish peroxidase, 1:2000 at 4 °C, visualization was achieved using 3,3'-diaminobenzidine as substrate.

Measurements of marker enzymes and proteins

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 no. 3359 and 3344 respectively). The activity of (Na⁺ + K⁺)-ATPase (EC 3.6.1.3) was measured by the coupled optical assay at room temperature as described by Berner and Kinne (1976). Proteins were determined by the method of Bradford (1976).

Statistical evaluation

The data are shown as means $\pm$ S.E.M. For statistical analyses Student's *t* test for unpaired samples at the level of significance of 0.05 was used.

Table 1 Concentrations of DAG and inositol lipids in kidney cortex, BBM and BLM

Each value is from five independent experiments and each measurement of lipid concentration was performed in duplicate.

	Content (pmol/mg of protein)			
	DAG	PtdInsP ₂	PtdInsP	PtdIns
Kidney cortex	207 $\pm$ 14	637 $\pm$ 62	1150 $\pm$ 39	15.7 $\pm$ 0.2
BBM	327 $\pm$ 35	1875 $\pm$ 102	2728 $\pm$ 205	45.1 $\pm$ 2.3
BLM	312 $\pm$ 21	911 $\pm$ 73	1822 $\pm$ 201	30.2 $\pm$ 0.9

RESULTS

As shown in Table 1, there is no significant difference between DAG concentrations in BBM and BLM, whereas concentrations of inositol lipids are significantly higher ($P < 0.01$) in BBM than in BLM. The values for PtdIns concentration in BLM are almost identical with those measured by Bode et al. (1976) and Hise et al. (1984), although we employed enzymic measurement of lipid concentration, whereas their measurements were based on determination of total phospholipid phosphorus. On the other hand, somewhat higher concentration of PtdIns were observed in BBM than were reported by the above-mentioned authors, which can be explained by the different methods of BBM isolation; the Mg/EGTA precipitation method yields very pure membrane preparations (Biber et al., 1981).

Previous observations that plasma from uninephrectomized rats stimulates phospholipase C in intact renal cortical slices (Kukolja et al., 1986; Banfić and Kukolja, 1988; Banfić, 1989) are in agreement with the present data shown in Figure 1. Increases in DAG concentration in BBM could be observed 10 min to 3 h after unilateral nephrectomy, with a significant decrease only in PtdInsP₂ 10 min after the operation. The increase in DAG is greater than the loss of inositol lipids, and we assume that, as occurs in the plasma membrane in other tissues (Downes et al., 1989), some resynthesis of PtdInsP and PtdInsP₂ has taken place; however, this would not produce an experimentally detectable decrease in PtdIns. There might also be a contribution to DAG from other lipids, such as phosphatidylcholine (PtdCho) (Exton, 1990), although we were not able to demonstrate any

Figure 1 Time course of changes in (a) DAG, (b) PtdInsP₂, (c) PtdInsP and (d) PtdIns concentrations in BBM from the remaining kidney after unilateral nephrectomy (◆) and from the corresponding kidney in sham-operated animals (□)

Each point represents the mean $\pm$ S.E.M. from four different experiments in duplicate. Asterisks indicate significant difference ($P < 0.05$, Student's *t* test) from controls.

Figure 2 Time course of changes in (a) DAG, (b) PtdInsP₂, (c) PtdInsP and (d) PtdIns concentrations in BLM isolated from rat kidney cortical slices stimulated with IGF-II (10 nM) (◆).

Each point represents the mean ± S.E.M. from four different experiments in duplicate. Asterisks indicate significant difference (*P* < 0.05, Student's *t* test) from controls (□).

Figure 3 Effect of blood flow occlusion and reflow in one kidney on DAG concentration in (a) BBM and (b) BLM from the contralateral kidney

Blood flow through the left kidney was stopped by ligating the renal artery at zero time. The rats were killed either 15 min (■) or 45 min (■) later or the reflow was established by removing the ligature 15 min after the blood flow occlusion and the animals were killed 30 min thereafter (◆). Control animals (time 0) were sham-operated. Each point represents the mean ± S.E.M. from four separate membrane preparations in each group. *Significantly different from control values; †significantly different from value obtained by ligating the renal artery.

PtdCho breakdown by way of phospholipase C activation *in vitro* (Banfić et al., 1992). It is important to note that, during the entire experimental period (5 min to 48 h), no changes in either DAG or inositol lipid concentrations were observed in BLM prepared from the remaining kidney (results not shown).

As classical hormonal signals in the kidneys are mediated through BLM (Morel and Doucet, 1986), and as we failed to observe activation of phospholipase C in BLM after unilateral nephrectomy, we decided to check inositol lipid signalling by way of BLM using IGF-II, a well-known agonist which stimulates

Figure 4 Translocation of PKC to BLM and BBM

Plasma membranes were isolated as described in the Materials and methods section. Protein (50 μ l) was subjected to SDS/PAGE, transferred to nitrocellulose and probed with an anti-PKC antibody. Lane 1, BLM isolated 15 min after blood flow occlusion through the contralateral kidney; lane 2, control BLM; lane 3, BLM isolated 45 min after blood flow occlusion through the contralateral kidney; lane 4, BLM isolated 15 min after blood flow occlusion, which was followed by re-established blood flow for 30 min through the contralateral kidney; lane 5, BBM isolated 15 min after blood flow occlusion through the contralateral kidney; lane 6, control BBM; lane 7, BBM isolated 45 min after blood flow occlusion through the contralateral kidney; lane 8, BBM isolated 15 min after blood flow occlusion, which was followed by re-established blood flow for 30 min through the contralateral kidney; lane 9, BLM isolated after stimulation of renal cortical slices for 5 min with IGF-II (10 nM); lane 10, BBM isolated after stimulation of renal cortical slices for 5 min with IGF-II (10 nM).

inositol lipid signalling in canine BLM (Rogers and Hammerman, 1988). As shown in Figure 2, IGF-II stimulates an increase in DAG concentration, with a concomitant decrease in PtdIns P_2 and PtdIns P concentrations in BLM. In contrast, no changes in either DAG or inositol lipid concentrations were observed in BBM (results not shown). Identical results were obtained when we used the α_1 -agonist phenylephrine (0.1 mM) (results not shown), which has also been demonstrated to stimulate phospholipid metabolism in rat kidney cortical tubules (Wirthensohn et al., 1984).

In a previous paper (Salihagić et al., 1988), we have shown that stimulation of Na⁺/H⁺ exchange in BBM in one kidney was also induced by the occlusion of blood flow through the contralateral kidney and that Na⁺/H⁺ exchange returned to the control preoperative value when the reflow was established. Results from identically designed experiments are shown in Figure 3. At 15 min after the occlusion of blood flow through the left kidney by ligation of the left renal artery, the concentration of DAG in BBM from the right kidney significantly increased, but no increase in DAG concentration could be observed in BLM. If the ligature was kept in place for an additional 30 min, the DAG concentration remained significantly elevated. Consequently, we were able to mimic the increase in BBM DAG concentration seen after unilateral nephrectomy (Figure 1). However, if blood flow through the left kidney was occluded for 15 min and then re-established by removal of the ligature, 30 min later the DAG concentration in the renal luminal membrane from the contralateral kidney did not differ from that in the control BBM. These results indicate that the stimulation of phospholipase C in BBM is caused by a rapid and reversible mechanism.

The simplest interpretation of the data described above is that unilateral nephrectomy and blood occlusion through the contralateral kidney both induce phospholipase C-catalysed hydrolysis of inositol lipids in BBM, whereas 'classical hormones' do the same thing in BLM. Phospholipase C activation is followed by

translocation of PKC from the cytosol to membranes, a process caused primarily by the generation of DAG, but probably also requiring increased intracellular Ca²⁺ (Nishizuka, 1992). It is intriguing that phospholipase C-mediated cell stimulation might be exerted through BBM or BLM and could involve translocation of PKC to the opposite side of the plasma membrane. We have investigated this using an anti-PKC antibody (one recognizing a consensus sequence common to α , γ and δ PKCs). As shown in Figure 4, blood occlusion and reflow through the left kidney does not induce any translocation of PKC to BLM in the right kidney (lanes 1–4), whereas blood occlusion through the left kidney for either 15 or 45 min (lanes 5 and 7) stimulates translocation of PKC (which has an M_r of about 80000) to BBM in the right kidney, compared with the control preoperative amount (lane 6). Translocation of PKC to BBM but not BLM of the remaining kidney was also observed when unilateral nephrectomy was performed 15, 30, 60 and 180 min before membrane isolation (Western blots not shown). Moreover, if the blood flow through the left kidney was occluded for 15 min and then re-established by removal of the ligature, 30 min later the amount of PKC in BBM in the right kidney returned to the control preoperative value (lane 8), showing that translocation of PKC is caused by a rapid and reversible mechanism. Finally, IGF-II induced translocation of PKC to BLM (lane 9) but not BBM (lane 10), demonstrating once again the difference between a classical hormonal signal and the signal produced by unilateral nephrectomy.

DISCUSSION

The results presented here show that in renal cells there is a spatial separation of the inositol lipid signalling system between BLM and BBM. Although specific binding of ¹²⁵I-labelled IGF-II was demonstrable in isolated BLM and BBM with equal binding capacities for each membrane (Hammerman and Rogers, 1987), our results corroborate the previous observation (Rogers and Hammerman, 1988) that IGF-II stimulates inositol lipid signalling only in BLM. It is important to note that Rogers and Hammerman (1988) measured phospholipase C activation in native membranes incubated with IGF-II, whereas we isolated BLM after we had incubated intact renal cortical slices with an agonist. In preliminary experiments (H. Banfić, unpublished work), we were unable to observe any phospholipase C activation on stimulation of isolated membranes with IGF-II, although procedures for isolation of BLM and BBM were almost identical with those described by Rogers and Hammerman (1988). The difference probably lies in the different animal species used in the experiments (canine versus rat kidney). It can be argued that, during isolation of rat renal cell membranes, the delicate interrelationship between receptor, G-protein and phospholipase C is broken. Consequently, these proteins are not functionally coupled in isolated membranes. On the other hand, our observation extended the results of Rogers and Hammerman (1988), as we were able to demonstrate PKC translocation, a process caused primarily by the generation of DAG (Nishizuka, 1992), to BLM but not to BBM.

The stimulus (or stimuli) that causes compensatory renal growth is poorly understood, but, in principle, two hypotheses exist. The premise of the 'work hypothesis' is that the increased work load after uninephrectomy may be the triggering signal for renal hypertrophy, whereas according to the 'renotropic hypothesis' a humoral factor is responsible for it (Fine, 1986; Wesson, 1989). Our recent study performed *in vitro* favours the renotropic hypothesis by demonstrating that plasma from uni-

nephrectomized rats stimulates phospholipase C activation in renal cortical slices (Banfić and Kukolja, 1988). In the present study, *in vivo* activation of phospholipase C was demonstrated, which, together with the perfect temporal overlapping of increased phospholipase C activity *in vitro* and *in vivo* (10 min to 3 h after unilateral nephrectomy), further suggests the existence of a humoral factor responsible for the initiation of compensatory renal growth.

It is surprising that signalling is mediated through BBM; although the presence of phospholipase C in rat kidney BBM has been known for some time (Schwartz et al., 1983), until recently it was not known whether it could be functionally activated by any receptor on the apical side of the cell (Reskhi et al., 1991). Sustained elevated levels of DAG in BBM of the remaining kidney after uninephrectomy suggests that there should be other sources of DAG besides inositol lipids. Although we were not able to demonstrate any PtdCho breakdown *in vitro* by way of phospholipase C activation (Banfić et al., 1992), increased phospholipase D activity was observed in the cortex of the remaining kidney (Gatalica et al., 1993), the time course of activation being a little behind that of phospholipase C activation observed in this paper. Several lines of evidence suggest that PtdCho is hydrolysed by phospholipase D, resulting in the formation of phosphatidic acid, which is then converted into DAG by the action of phosphatidic acid phosphomonoesterase (Nishizuka, 1992). The process that occurs after phospholipase C activation and generation of DAG is translocation of PKC from the cytosol to membranes (Nishizuka, 1992), which also happens in BBM after unilateral nephrectomy or blood occlusion through the contralateral kidney. Increased PKC activity in BBM of the remaining kidney has also been observed 24 and 48 h after unilateral nephrectomy (Hise and Mehta, 1989), and, although DAG concentration at these time points is not changed, it is known that there are other activators of PKC besides DAG (Nishizuka, 1992). The metabolites of phospholipase A₂ activation might be of particular interest, as it has been observed both *in vitro* (Banfić and Gatalica, 1989) and *in vivo* (Logan et al., 1986) that they influence compensatory renal growth. Another important possibility that emerges from separate inositol lipid signalling systems in BBM and BLM is that renal cells can easily distinguish from which side they are stimulated, and, despite the same general signalling system used, can probably respond differently. This might be even more important when a dual messenger system is used, as has been demonstrated for parathyroid hormone (Reskhi et al., 1991), or during initiation of compensatory renal growth (Banfić, 1990).

The experiments involving uninephrectomy and temporary occlusion of blood flow through the renal artery followed by reflow clearly indicate the presence of a mechanism that regulates fast and reversible changes in Na⁺/H⁺ antiporter activity in the luminal membrane of proximal tubular cells (Salihagić et al., 1988). In the light of the fast and reversible changes in phospholipase C activity and PKC translocation to BBM under the experimental conditions shown here, this strongly favours the possibility that activation of exchanger is caused by PKC-mediated protein phosphorylation, as has already been shown in solubilized brush-border membrane proteins (Weinman et al., 1990) and primary cultures of renal proximal tubular cells (Hise and Mehta, 1989; Horie et al., 1992).

Recently, it was demonstrated that denervation inhibits the early increase in Na⁺/H⁺ exchange after uninephrectomy but does not suppress hypertrophy. This suggests that a renorenal reflex arc mediates the increase in antiporter activity through sympathetic nerves, but dissociates renal growth from the Na⁺/H⁺ antiport activation (Mackovic-Basic et al., 1992). This means

that Na⁺/H⁺ exchanger activation does not have a crucial role in growth of renal cells as has been reported for some other cell types (Grinstein and Rothstein, 1986; Moolenaar et al., 1983; Schwartz et al., 1983; Seiffer and Aronson, 1986). Proximal tubules have adrenergic receptors on their plasma membrane and are richly innervated (Sundaresan et al., 1987). Although activation of α_1 -receptors stimulates phosphoinositide hydrolysis in rat cortical tubules (Wirthensohn et al., 1984) and in MDCK cells (Slivka and Insel, 1987), the present results clearly demonstrate that signalling during initiation of compensatory renal growth is not mediated by way of α_1 -receptor activation, as phenylephrine activates phospholipase C in BLM, whereas unilateral nephrectomy and blood flow occlusion stimulate phospholipase C in BBM. Similar conclusions can be made about the functioning of renal dopaminergic nerves, as it is known that dopamine-1 receptors that are coupled to phospholipase C are only located in BLM (Felder et al., 1989a,b). Nevertheless, renal nerves may be involved in the signalling processes to the remaining kidney via the afferent arc of neurocirculatory reflex; 30 min after unilateral nephrectomy, an increase in peripheral plasma concentration of γ -melanocyte-stimulating hormone-like peptide from the pituitary was observed (Lin et al., 1985, 1987). Thus this peptide can be added to the long list of humoral factors that might be involved in the control of transport processes and growth of kidney cells (Fine, 1986; Morel and Doucet, 1986; Segal and Fine, 1989; Wesson, 1989).

An increase in plasma concentration of atrial natriuretic peptide 2 h after unilateral nephrectomy has also been reported, together with an increase in urinary cyclic GMP excretion (Valentin et al., 1990). Although it is known that unilateral nephrectomy increases cyclic GMP concentration in the renal cortex of the remaining kidney (Dicker, 1977; Schlondorff and Weber, 1976), there is little chance that atrial natriuretic peptide causes inositol lipid signalling in BBM, as it is known that this peptide mainly affects the distal portion of the nephron (De Wardener and Clarkson, 1985), whereas, using the above-mentioned methods for the preparation of BBM and BLM, the majority of the plasma membrane is found to originate from the proximal tubule. Also, to our knowledge, there has been no report of inositol lipid signalling in the renal cells on stimulation with atrial natriuretic peptide. Moreover, inositol lipid signalling precedes any increase in cyclic GMP concentration both *in vivo* [present results compared with the data of Dicker (1977) and Schlondorff and Weber (1976)] and *in vitro*, where it was observed that an increase in cyclic GMP depends on extracellular Ca²⁺ (Banfić and Kukolja, 1988), suggesting that an increase in cyclic GMP concentration is a consequence of inositol lipid signalling.

To summarize, the present results demonstrate that, in renal cells, there is a spatial separation of the inositol lipid signalling system between BLM and BBM, and that uninephrectomy causes activation of phospholipase C only in BBM. It seems that the stimulus factor for compensatory renal growth is present in tubular fluid and might be excreted in urine. Indeed, it has been observed that reinfusion of half the urine output in rat stimulates renal growth, and this is not diminished by dialysis of the urine before its reinfusion, suggesting that the renal growth stimulator(s) in urine is not readily dialysable (Harris and Best, 1979). Unfortunately, one peptide of particular interest, epidermal growth factor (EGF), which can be isolated from urine in its low- and high-*M_r* forms (Nexo et al., 1990) and is largely synthesized in the kidney (Salido et al., 1991) and might act within it as a paracrine agent, is not a candidate to explain the effects observed in this paper, as it is known that its major site of action is the collecting tubule, particularly the inner medullary region (Breyer et al., 1988; Harris, 1989), and only there can it stimulate

phosphoinositide hydrolysis (Teitelbaum et al., 1990). Moreover, although increased distal nephron EGF content was observed in the remaining kidney (Miller et al., 1992), there is no specific binding of EGF to rat kidney apical cortical membranes (Behrens et al., 1989) and, in renal cortical slices, EGF is not capable of stimulating phospholipase C (Gatalica and Banfić, 1988). Finally, although the biochemical nature of the stimulus that initiates compensatory renal growth is still unknown, experiments with blood flow occlusion and reflow through renal artery clearly indicate the presence of a fast and reversible mechanism of changes in phospholipase C activity and PKC translocation to BBM.

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