Endothelin Mediates Phospholipase C Stimulation in the Proximal Tubule During Initiation of Compensatory Renal Growth in Adult Rats

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Aim. Mechanisms that initiate compensatory renal growth following unilateral nephrectomy are incompletely understood. An early event following unilateral nephrectomy is the activation of phospholipase C in the apical membrane of the proximal tubule, mediated by an unknown agonist. We tested the hypothesis that endothelin is responsible for the stimulation of phospholipase C in rat proximal tubule following unilateral nephrectomy.

Methods. Compensatory renal growth was induced in adult male rats by unilateral nephrectomy. 1,2-Diacylglycerol, a product of phospholipase C activation, was measured in renal cortical slices and isolated proximal tubules, respectively, 20 min following unilateral nephrectomy, or after incubation of the slices or proximal tubules with plasma from unilaterally nephrectomized or sham-operated rats.

Results. Twenty min following unilateral nephrectomy, an increase in 1,2-diacylglycerol concentration occurred in the renal cortex. Bosentan, a nonselective endothelin receptor antagonist, as well as an anti-endothelin-1 antibody administered intravenously, completely inhibited this 1,2-diacylglycerol accumulation in renal cortex. Incubation of renal cortical slices with plasma from unilaterally nephrectomized or bilaterally nephrectomized rats, stimulated 1,2-diacylglycerol production in isolated proximal tubule apical membranes. Again, bosentan prevented the increase evoked by incubation with plasma from unilaterally nephrectomized rats. Finally, concentration of endothelin-1 increased in renal cortex in response to unilateral nephrectomy.

Conclusion. These results make evident the role of endothelin in stimulation of phospholipase C in proximal tubule following unilateral nephrectomy, suggesting participation of the endothelin system during the initiation of the compensatory renal growth.

Key words: endothelin-1; endothelin-2; endothelin-3; endothelin receptors; kidney; kidney tubules; nephrectomy

Mechanisms that initiate compensatory renal growth are incompletely understood. Proximal tubule is the nephron segment whose hypertrophy contributes mainly to the final compensatory renal growth (1). In vivo studies demonstrated transient activation of phospholipase C and translocation of protein kinase C immediately after unilateral nephrectomy to the proximal tubule brush-border membrane in the rat (2). In addition, plasma obtained from rats early after unilateral nephrectomy was shown to stimulate phospholipase C and phospholipase D in renal cortex (2,3). These results suggest appearance of a humoral factor immediately after unilateral nephrectomy, which stimulates phospholipase C exclusively in proximal tubule apical membrane. We found that endothelin-1 and endothelin-2 stimulated phospholipase C exclusively in brush-border membrane through the activation of the endothelin ET \(_2\) receptors (4). In the proximal tubule, endothelin-1 regulates Na\(^+\)/H\(^+\) exchanger (5), as well as Na\(^+\)/Pi and Na\(^+\)/HCO\(_3\) cotransporters (6,7) and stimulates growth of the proximal tubule cells in culture (8). Moreover, observations that proximal tubules produce endothelin-1 (8) and that endothelin-2 is produced by renal adeno-
carcinoma cells (9), make participation of endothelins in renal growth responses possible.

This study was designed to examine the role of endothelin in the phospholipid signaling during initiation of the compensatory renal growth following unilateral nephrectomy in vitro and in vivo in adult rats. The present results suggest that endothelin mediates activation of phospholipase C in the proximal tubule apical membrane early after unilateral nephrectomy.

Material and Methods

Material

Endothelin-1 (human, porcine) and polyclonal anti-endothelin-1 antibody were made by Sigma (St. Louis, MO, USA). Bosentan was a gift from Dr Martine Clozel, F. Hoffmann-La Roche, Basel, Switzerland. Pyruvate kinase, lactate-dehydrogenase and phosphoenolpyruvate were from Boehringer (Mannheim, Germany). \(^{32}\)P-ATP (3000 Ci/mmol) was from Amersham (Arlington Heights, IL, USA). All other chemicals used were of p.a. grade purchased commercially.

Animals

Studies were performed on male Wistar rats aged 3-4 months, bred at the Departmental facility. Rats were ether-anesthetized and
right nephrectomy was performed by dorsolateral approach. For bi-
lateral nephrectomy bilateral dorsolateral incision was done and
both kidneys were removed at the same time. For sham nephre-
ctomy, the right kidney was exposed, gently manipulated and the
perirenal fat was removed. In experiments with ligation of the ureter,
midadominal incision was made and the right ureter was exposed and
ligated just below the renal hilus. In sham operated rats, the ureters
was exposed without ligating it.

In Vivo Experiments
For the in vivo experiments, four rats in each experimental
group were used. A nonselective ET_A receptor antagonist bosentan
(10) (30 mg/kg), or the vehicle, as well as an anti-endothelin anti-
body were administered as bolus 10 min prior to nephrectomy into
internal jugular vein. Twenty min after nephrectomy (or sham
nephrectomy) animals were killed and left kidney was removed and
placed into ice cold phosphate-buffered saline (PBS). Kidneys were
decapsulated and the cortex was dissected and homogenized for 2
min in buffer containing (in mmol/L): 300 mannitol, 5 EGTA, 10
HEPES/Tris, pH 7.4. Cortical tissue suspension was centrifuged in a
refrigerated centrifuge at 4,800 G for 15 min. The pellet was dis-
carded and the supernatant was further centrifuged at 10,000 G for
10 min, after which the pellet was stored at -75°C prior to further
processing.

In Vitro Experiments
Twenty min after the rats (four in each group) were sham oper-
ated, uninephrectomized, or bilaterally nephrectomized they were
anesthetized again, and over the period of 2 min 4 mL of blood was
withdrawn from the internal jugular vein into heparinized glass
tubes. Blood was immediately centrifuged in a refrigerated centri-
fuge to obtain plasma. Plasma was kept on ice and was used for the
experiments immediately. Renal cortical slices from intact animals,
killed by cervical dislocation, were prepared from decapsulated kid-
ey using Stadie-Riggs microtome. Two slices were one sample. The
slices were placed into preoxygenated Hank's solution contain-
ing 25% v/v plasma and were incubated for 7 min. This plasma vol-
ume ratio and time of incubation were previously shown to give the
best response (11). When indicated, bosentan (10-5 mol/L) was
added 10 min prior to plasma where indicated. When endothelin-1
was added to the slices together with plasma from unilaterally nephrecto-
mized rats, the response was only partially augmented (816±53 pmol/mg protein, Fig. 1). An increase in
1,2-diacylglycerol was obtained with plasma from bi-
laterally nephrectomized rats as well (Fig. 1). Bosentan
(105 mol/L) completely prevented this increment in
1,2-diacylglycerol in response to plasma from unilater-
ally nephrectomized rats, not affecting basal 1,2-di-
acylglycerol level in brush-border membrane (305±38
vs 313±31, not significant, Fig. 1). During the in vivo
response to acute unilateral nephrectomy, 20 min
unilateral nephrectomy, but not sham nephrectomy,
increased 1,2-diacylglycerol concentration in the re-
nal cortex (490±14 vs 207±30 pmol/mg protein,
p<0.01, Fig. 2). Pretreatment with bosentan (30
mg/kg IV) had no effect on 1,2-diacylglycerol level in
sham operated animals (data not shown), but pre-
vented any response to the unilateral nephrectomy
(217±26 pmol/mg of protein, not significant vs sham
unilateral nephrectomy, Fig. 2). Similar effect was ob-
tained with IV administration of an anti-endothelin-1
antibody prior to unilateral nephrectomy (195±18
pmol/mg of protein, p<0.01 vs sham, Fig. 2).

Since these results implicate the endothelin sys-
...in the initial response to the acute unilateral
Concentration of big endothelin-1/endothelin-1, determined by a radioimmunoassay, in the renal cortex was determined 20 min after unilateral nephrectomy (UNI), or sham nephrectomy (SHAM). Bosentan (bos.) or an anti-endothelin-1 antibody (anti-ET-1) were administered intravenously 10 min prior to nephrectomy where indicated. N=4, asterix indicates p<0.01 vs. sham nephrectomy (F=154.2, R²=0.975); one-way ANOVA with post-hoc Newman-Keuls multiple comparison test.

**Figure 2.** Concentration of 1,2-diacylglycerol in the renal cortex was determined 20 min after unilateral nephrectomy (UNI), or sham nephrectomy (SHAM). Bosentan (bos.) or an anti-endothelin-1 antibody (anti-ET-1) were administered intravenously 10 min prior to nephrectomy where indicated. N=4, asterix indicates p<0.01 vs. sham nephrectomy (F=154.2, R²=0.975); one-way ANOVA with post-hoc Newman-Keuls multiple comparison test.

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**Figure 3.** Concentration of big endothelin-1/endothelin-1, determined by a radioimmunoassay, in the renal cortex from sham nephrectomized (SHAM) and unilaterally nephrectomized rats (UNI) 20 min following nephrectomy. N=4, asterix indicates p<0.01 vs. sham nephrectomy (F=154.2, R²=0.975); one-way ANOVA with post-hoc Newman-Keuls multiple comparison test.

Compensatory renal growth is a process of adaptation of the remaining kidney to the increased functional demand following unilateral nephrectomy. Mechanisms leading to the renal hypertrophy are incompletely understood. This applies particularly to the events during the initiation of compensatory renal growth. Previous studies demonstrated an early activation of the phospholipid signaling cascade in the cortex of the remaining kidney (11). Both in vivo and in vitro (following stimulation of cortical slices with the plasma obtained from unilateral nephrectomy rats), there is a time-dependent increase in 1,2-diacylglycerol formation in the proximal tubule apical membrane, paralleled with inositol-phosphate formation, as well as an activation of apical Na’/H+ exchanger (14). This suggests an action of a phospholipase C-stimulating hormone in the proximal tubule during the initiation of the compensatory renal growth. Such a confinement of 1,2-diacylglycerol production and phosphokinase C translocation to the apical membrane is somewhat unexpected, since the majority of agonists that stimulate phospholipase C in the proximal tubule act through the receptors located in the basolateral membrane, or, as is the case with AT1 receptors for angiotensin II, both apical and basolateral membrane are the sites (15). However, in our recent study, we have shown that endothelin ET₆ receptor-mediated phospholipase C signaling (1,2-diacylglycerol production and subsequent PKC translocation) occurred exclusively in the apical membrane of the rat proximal tubule. This finding raised the possibility that endothelins may be responsible for the signaling during the initiation of the compensatory renal growth. However, due to the lack of specificity of the antibody used in the radioimmunoassay in the present study, it is difficult to ascribe the observed response in 1,2-diacylglycerol to a particular endothelin isoform. However, the involvement of endothelin-3 seems unlikely, because it would lead, at least in the in vitro experiments, to the activation of phospholipase C in basolateral membrane as well (4). What is the source of endothelin following unilateral nephrectomy? Since plasma from bilaterally nephrectomized rats stimulated phospholipase C and bosentan inhibited 1,2-diacylglycerol production both in vivo and in vitro, it was either circulating endothelin, or endothelin released from renal cortical tissue (acting in an autocrine manner) that stimulated phospholipase C. It is also possible that the upregulation and/or increased sensitivity of ET₆ receptors play a role in endothelin-mediated signaling after unilateral nephrectomy. A possible role of endothelin in compensatory renal growth is in concert with the results of Nakamura et al (16) who showed that three hours after the nephrectomy, endothelin-1 and ET₆ mRNA
levels in the renal cortex increased significantly and then decreased gradually to the control level after 24 h; in contrast, endothelin-3 and ETα receptor mRNA levels demonstrated little change. Compensatory hypertonphy of the remaining kidney is mainly due to the hypertrophy of the proximal tubule (1). Various agonists have been implicated as mediators of compensatory renal growth, but none has been proved to be causative. For example, compensatory renal growth following unilateral nephrectomy occurred in the absence of measurable changes in the components of the renin-angiotensin system (17), as well as insulin-like growth factor system (18). Following nephrectomy, no enhancement of the intensity of immunostaining for insulin-like growth factor-1 was observed in the kidneys of nephrectomized rats until 5 post-nephrectomy days (19). Hepatocyte growth factor is another potent renal tubular cell growth factor (20). However, the active form of hepatocyte growth factor was not detected after unilateral nephrectomy (21). In addition, hepatocyte growth factor stimulates basolateral phospholipase C in the rat proximal tubule (22), which distinguishes it from the signal in compensatory renal growth. Pulsatile growth hormone release was markedly elevated 24 h after unilateral nephrectomy and administration of a growth hormone-releasing factor antagonist significantly suppressed renal growth 48 h post uninephrectomy (23). It remains to be explored whether there is a link between endothelin and growth hormone axis in the compensatory renal growth. Early functional events following unilateral nephrectomy are enhanced natriuresis and diuresis. Similar contralateral diuretic and natriuretic response can be seen following unilateral occlusion of the ureter, which increases ipsilateral renal afferent nerve activity and activates renal mechnoreceptors (24). In both cases there is an increase in plasma concentration of α-melanostimulating hormone. In the uninephrectomized rats immunized against γ-melanostimulating hormone, post-unilateral nephrectomy natriuresis was blunted (25). In the present study unilateral ureteral ligation did not affect cortical 1,2-diaclyglycerol level in either ipsilateral, or contralateral kidney, suggesting that mechanical stretch did not mediate endothelin release and/or action. Similarly to the angiotensin II, endothelin-1 biphasically affects proximal tubule sodium and water reabsorption (5), stimulating it in lower and inhibiting it in higher concentrations. Thus, endothelin might mediate proximal component of the natriuretic/diuretic response to unilateral nephrectomy. Could endothelin also be a candidate for a renotropin? Endothelins can affect growth responses of the proximal tubule. In the primary culture of human proximal tubule cells endothelin-1 was mitogenic through ETα receptors (8). Interestingly, ETα receptor stimulation augmented endogenous production of endothelin-1, suggesting positive autocrine loop (8). Since renal adenocarcinoma cells synthesize endothelin-2 (9), the participation of endothelin-2 in the renal cell growth seems possible. The possibility that endothelin-2 plays a role in the compensatory renal growth as well remains to be tested.

In conclusion, the present study provides evidence for the role of endothelin in the stimulation of phospholipase C in the proximal tubule during initiation of the compensatory renal growth following unilateral nephrectomy in the adult rat. However, long-term experiments with the use of endothelin receptor antagonists are required for the final conclusion about the role of endothelins in the functional adaptation and hypertrophy of the remaining kidney.

Acknowledgment

This work was supported by the Republic of Croatia Ministry of Science and Technology Grant No. 108013 to Dr H. Banfić.

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