Kidney International, Vol. 54 (1998), pp. 38–47

Obstructive nephropathy in the neonatal rat is attenuated by epidermal growth factor

ROBERT L. CHEVALIER, SHARAD GOYAL, JENNIFER T. WOLSTENHOLME, and BARBARA A. THORNHILL

Department of Pediatrics, University of Virginia, School of Medicine, Charlottesville, Virginia, USA

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Background. Obstructive nephropathy is a primary cause of renal failure in infancy. Chronic unilateral ureteral obstruction (UUO) in the neonatal rat results in reduced renal expression of epidermal growth factor (EGF), renal tubular epithelial (RTE) cell apoptosis and interstitial fibrosis. We wished to determine whether these changes could be prevented by exogenous administration of EGF.

Methods. Thirty-three Sprague-Dawley rats underwent UUO within the first 48 hours of life, and received daily injections of either EGF (0.1 mg/kg/day) or saline (control) for the following seven days, after which obstructed and intact opposite kidneys were removed for study. These were compared to 11 sham-operated rats that received either no injections, EGF injections, or saline injections. Renal cell proliferation was determined by proliferating cell nuclear antigen, apoptosis was measured by the TUNEL technique, and the distribution of vimentin, clusterin, transforming growth factor-beta 1 (TGF-β1), and α-smooth muscle actin were determined by immunohistochemistry. Tubular dilation, tubular atrophy, and interstitial collagen deposition were quantitated by histomorphometry.

Results. Compared to controls, EGF treatment increased RTE cell proliferation in the obstructed kidney by 76%, decreased apoptosis by 80%, and reduced vimentin, clusterin and TGF-β1 immunostaining (all $P < 0.05$). EGF treatment reduced tubular dilation by 50%, atrophic tubules by 30%, and interstitial fibrosis by 50% (all $P < 0.05$). There was no significant effect of EGF on renal alpha smooth muscle actin distribution. There was no effect of saline or EGF injections on kidneys from sham-operated rats for any of the parameters studied.

Conclusions. We conclude that EGF stimulates RTE cell proliferation and maturation and reduces apoptosis in the neonatal rat kidney subjected to chronic UUO. These effects may contribute to the reduction in tubular dilation, tubular atrophy, and interstitial fibrosis. By preserving renal development, administration of EGF attenuates the renal injury resulting from chronic UUO.

Congenital obstructive nephropathy is a primary cause of renal failure in infancy and childhood [1]. Renal failure results from the effects of urinary tract obstruction to delay growth and maturation of the developing kidney [2]. Kidneys from midtrimester human fetuses with congenital urinary tract obstruction have reduced nephrogenesis, cystic changes, and interstitial fibrosis [3]. We have characterized a model of obstructive nephropathy in the neonatal rat, a species in which only 10% of nephrons are present at birth, and nephrogenesis is complete by 7 to 10 days of age [4]. This period is analogous to that of the midtrimester human fetus, during which the major features of obstructive nephropathy evolve [3]. Unilateral ureteral obstruction (UUO) during the first 48 hours of life in the rat markedly impairs renal growth and delays renal maturation [5]. In addition, renal cell proliferation is decreased, while apoptosis (programmed cell death) is increased in the developing obstructed kidney [6]. The end result of chronic UUO in the neonatal rat is tubular atrophy and diffuse interstitial fibrosis [7].

We have demonstrated that one of the consequences of chronic UUO in the neonatal rat is a marked suppression of expression of renal prepro-epidermal growth factor (EGF) mRNA [5]. This is reflected by a suppression of renal tubular immunoreactive EGF in 14-day-old rats subjected to UUO [7]. EGF is a peptide growth factor expressed in the rat kidney beginning at approximately the third postnatal day, and with steadily increasing expression during the first month of life [5]. EGF has been shown to act as a potent mitogen for renal tubular epithelial (RTE) cells [8]. In addition, exposure of the normally developing metanephric kidney to EGF significantly reduces the rate of endogenous apoptosis that contributes to renal remodeling [9]. Moreover, short-term (24 to 72 hr) administration of EGF to adult rats with UUO increases mitoses and reduces apoptosis in the obstructed kidney [10].

There are a number of parallels between the response of the developing kidney to UUO, and the renal changes resulting from cystic kidney disease, as well as the renal response to ischemia. All are characterized by an activation of the renin-angiotensin system, tubular dilation, tubular atrophy, activation of tubular apoptosis, and tubular expression of clusterin, a dimeric glycoprotein expressed in a variety of forms of renal injury [7]. In addition, expression of EGF is reduced in both cystic and ischemic renal disease [11, 12]. A recent study has shown that exogenous EGF can

Key words: cell proliferation, apoptosis, vimentin, clusterin, tubular atrophy, interstitial fibrosis.

Received for publication July 30, 1997
and in revised form January 30, 1998
Accepted for publication January 30, 1998

significantly improve glomerular filtration rate, reduce apoptosis, and reduce tubular dilation in mice with experimental cystic kidney disease [13]. Moreover, administration of exogenous EGF to rats with ischemic nephropathy also ameliorates recovery by improving cell regeneration and repair [14]. We wished to test the hypothesis that prolonged exogenous administration of EGF to neonatal rats could maintain renal development and attenuate the interstitial fibrosis and tubular atrophy resulting from chronic UUO. The present study extends the observations of 24 to 72 hours administration of EGF to adult rats with UUO [10] by examining the long-term consequences of EGF on the neonatal obstructed kidney. The results show a dramatic effect of exogenous EGF to attenuate the injurious effects of UUO on the developing kidney.

METHODS

Animal preparation

Forty-four newborn Sprague-Dawley rats were subjected to complete left ureteral obstruction or sham-operation within the first 48 hours of life. Under general anesthesia with halothane and oxygen, the distal ureter was exposed and ligated (or left untouched) through a small abdominal incision with a silk suture. The incision was closed in a single layer and coated with collodion. The animals were then allowed to recover from anesthesia and were returned to their mothers. One day after surgery, 20 rats were injected subcutaneously with EGF (Upstate Biotechnology, Lake Placid, NY, USA) for seven days at a daily dose of 0.1 mg/kg body wt. Twenty rats were injected subcutaneously with saline vehicle, to serve as controls. An additional group of four sham-operated rats received no injections, to control for the effect of injection. Rats were sacrificed eight days after operation, and kidneys were removed, decapsulated, weighed, and processed for histologic study.

Morphometric analysis

Kidneys were fixed in 10% phosphate buffered formalin, dehydrated through graded alcohol and xylene, embedded in paraffin, sectioned at 5 μm, and stained with Masson trichrome stain or periodic acid Schiff (PAS).

Renal interstitial collagen distribution. Sections were examined by light microscopy under $\times$400 magnification, and ten nonoverlapping fields containing cortex and medulla were examined for each kidney. Using image analysis software (Mocha 1.2; Jandel Scientific, San Rafael, CA, USA), digital images of the Masson trichrome stained sections were superimposed on a grid, and the number of grid points overlapping interstitial blue-staining collagen was recorded for each field.

Tubular luminal area. Sections were examined under $\times$400 magnification, and ten nonoverlapping fields were scanned for each kidney. Tubular luminal areas were recorded for each field of the Masson trichrome stained sections.

Tubular atrophy. PAS-stained kidney sections were examined under $\times$400 magnification, and the number of atrophic tubules was quantitated in 10 nonoverlapping fields. Atrophic tubules were identified by thickened basement membranes.

Immunohistochemistry

Immunohistochemical localization was performed on formalin fixed, paraffin-embedded tissue using the avidin-biotin immunoperoxidase method (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA, USA). The primary antibodies used were monoclonal mouse anti-rat proliferating cell nuclear antigen (PCNA) diluted 1:150 in phosphate buffered saline (PBS; Vector Laboratories), monoclonal mouse anti-human vimentin (1:650; Sigma Chemical Co., St. Louis, MO, USA), monoclonal rabbit anti-rat clusterin (1:50; Upstate Biotechnology, Lake Placid, NY, USA), monoclonal anti-α-smooth muscle-specific actin (1:400; Sigma Chemical Co.), and polyclonal rabbit anti-human TGF-β1 (1:100; Promega, Madison, WI, USA). Selected serial sections were treated for 30 minutes with biotinylated forms of Dolichos biflorus agglutinin (1:400) or Lotus tetragonolobus lectin (1:25; Vector Laboratories) to identify collecting ducts or proximal tubules, respectively. Sections were counterstained with Gill's #2 hematoxylin, dehydrated and mounted for examination by light microscopy. As a negative control, the primary antiserum was replaced by nonimmune rabbit serum and no staining occurred.

Apoptotic nuclei. Apoptotic nuclei were labeled using the Tdt uridine-nick-end-label (TUNEL) technique (Apoptag; Oncor, Gaithersburg, MD, USA). Histologic sections were digested for eight minutes in proteinase K, 20 μg/ml, and washed in distilled water. Endogenous peroxidase was quenched with 2% hydrogen peroxide in PBS methanol for 10 minutes, and samples were exposed to digoxigenin11-dUTP using terminal deoxynucleotidyl transferase (Tdt) (1:5) for one hour at 37°C. Samples were rinsed in PBS, blocked with 1% globulin-free bovine serum albumin for 15 minutes, treated with anti-digoxigenin peroxidase FAB fragments (Oncor), and incubated for 30 minutes at room temperature. Color was developed using 0.07% diaminobenzidine and 0.02% hydrogen peroxide for five minutes. Sections were counterstained with hematoxylin, dehydrated in graded alcohol, and examined by light microscopy.

Kidneys were examined by light microscopy at $\times$400 magnification. Ten nonoverlapping fields were scanned and for sections stained for PCNA or apoptosis, the number of labeled tubular and interstitial cells was counted in each field. A semiquantitative method was used to examine the interstitial cellular staining of α-smooth muscle actin and vimentin, and the tubular cell staining of clusterin, TGF-β1, and vimentin. A scale from 1 (no staining) to 3 (maximal

Table 1. Characteristics of sham-operated rats

	No treatment	Saline	EGF
Number of rats	4	4	3
Body weight *(g)*	20.4 ± 0.4	21.2 ± 0.4	17.3 ± 0.6[a]
Left kidney/body wt	5.2 ± 0.1	5.1 ± 0.2	5.0 ± 0.2
Right kidney/body wt	5.2 ± 0.1	5.2 ± 0.2	5.0 ± 0.1
PCNA-staining tubular cells/field	79.8 ± 48.9	99.2 ± 21.5	86.8 ± 6.6
Apoptotic tubular cells/field	1.0 ± 1.0	2.0 ± 0.6	1.7 ± 1.7

Abbreviations are: EGF, epidermal growth factor; wt, weight; PCNA, proliferating cell nuclear antigen.

[a] $P < 0.05$ vs. No treatment and Saline

staining) was used to assess immunostaining in the kidney sections. For each antibody, the results were normalized to the score for the intact kidney of vehicle (saline) treated rats. All histomorphometry and immunohistochemical determinations were performed with the observer blinded to the treatment group.

Statistical analysis

Data are presented as mean ± standard error. Comparisons between groups were made using one-way analysis of variance followed by the Student-Newman-Kuels test. Comparisons between left and right kidneys were performed using the Student's *t*-test for paired data. Statistical significance was defined as $P < 0.05$.

RESULTS

The relative survival of rats receiving EGF was 79%, which did not differ from that of animals receiving saline vehicle (88%). Administration of EGF reduced body wt by 14% in rats undergoing UUO (14.7 ± 0.6 g vs. 17.2 ± 0.5 g, $P < 0.05$), and by 18% in rats undergoing sham operation (17.3 ± 0.6 vs. 21.2 ± 0.4 g, $P < 0.05$; Table 1). Daily saline vehicle injections did not affect body wt in sham-operated animals (Table 1). Kidney weight was proportional to body wt, such that the kidney/body wt ratio for control versus EGF groups was not different for either obstructed (6.1 ± 0.4 vs. 5.8 ± 0.2) or intact kidneys (6.1 ± 0.2 vs. 5.2 ± 0.4). There was no effect of injections or of EGF on kidney/body wt ratio in sham-operated rats (Table 1). Renal tubular epithelial cell proliferation, as measured by PCNA labeling, was reduced by ipsilateral UUO compared to the intact kidney (Figs. 1A, B and 2 A, B). However, administration of EGF increased RTE cell proliferation in the obstructed kidney and reduced proliferation in the intact opposite kidney (Fig. 2A). The sum of proliferating RTE cells for the combined left and right kidneys did not differ between groups (*P* not significant). Interstitial cell proliferation was increased in obstructed compared to intact kidneys ($P < 0.05$; Fig. 2B), but the proliferative activity was markedly less than that of RTE cells in either kidney (Fig. 2A). Administration of EGF reduced interstitial cell proliferation in the obstructed kidney, and did not affect cell proliferation in the intact kidney (Fig. 2B). There was no significant effect of saline or EGF injections on renal tubular cell proliferation in sham-operated rats (Table 1). There were only occasional PCNA-positive nuclei in the interstitium of sham-operated rats regardless of treatment (data not shown).

Ureteral obstruction stimulated RTE cell apoptosis. As illustrated in Figure 1C, brown-staining tubular cells manifest the characteristics of apoptosis, including condensed nuclei with cytoplasmic "blebs." As shown in Figure 2C, compared to the intact kidney, UUO increased the number of apoptotic RTE cells by over 30-fold ($P < 0.05$). Administration of EGF reduced the number of apoptotic RTE cells in the obstructed kidney by 80% (Figs. 1D and 2C). The number of apoptotic interstitial cells was also greater in the obstructed compared to the intact opposite kidney ($P < 0.05$), and EGF treatment reduced the population of apoptotic cells (Fig. 2D). There was no significant effect of saline or EGF injections on renal tubular cell apoptosis in sham-operated rats (Table 1). There were only occasional apoptotic nuclei in the interstitium of sham-operated rats regardless of treatment (data not shown).

Compared to the intact kidney, chronic UUO resulted in marked accumulation of interstitial collagen ($P < 0.05$; Figs. 1E and 2E). This was reduced by over 50% following the administration of EGF (Figs. 1F and 2E).

Vimentin is a microfilament protein expressed by mesenchymal, but not by normal, polarized epithelial cells. Compared to the intact kidney, in which vimentin was not expressed by tubular cells, UUO induced RTE cell vimentin immunostaining in scattered tubules (Fig. 3A). This increased expression of vimentin was significantly reduced by EGF administration (Fig. 4A).

Clusterin is a dimeric glycoprotein that is expressed by distal renal tubular cells in response to injury. Compared to the intact kidney, in which clusterin was not expressed by tubular cells, UUO induced clusterin immunostaining in scattered tubules (Fig. 3B). Tubular clusterin expression was significantly reduced by EGF administration (Fig. 4B).

Renal tubular cell expression of TGF-β1, a fibrogenic cytokine, was increased in the obstructed kidney (Fig. 3C). Immunoreactive TGF-β1 in the obstructed kidney was significantly reduced by EGF treatment (Fig. 4C).

Alpha-smooth muscle actin is a contractile protein normally expressed by vascular smooth muscle cells, as well as by interstitial cells during the first week of life in the rat [15]. Whereas interstitial expression of immunoreactive α-smooth muscle actin had disappeared by 10 days of life in the intact kidney (not shown), it was significantly increased by chronic UUO (Fig. 3D). While administration of EGF tended to reduce the expression of interstitial α-smooth muscle actin in the obstructed kidney, this was not statistically significant (Fig. 4D). As shown in Figures 3E and 4E, compared to the intact kidney, UUO caused marked dilation of a majority of tubules ($P < 0.05$). Administration of EGF reduced the degree of tubular dilation in the obstructed kidney by over 50% (Fig. 4E). As shown in

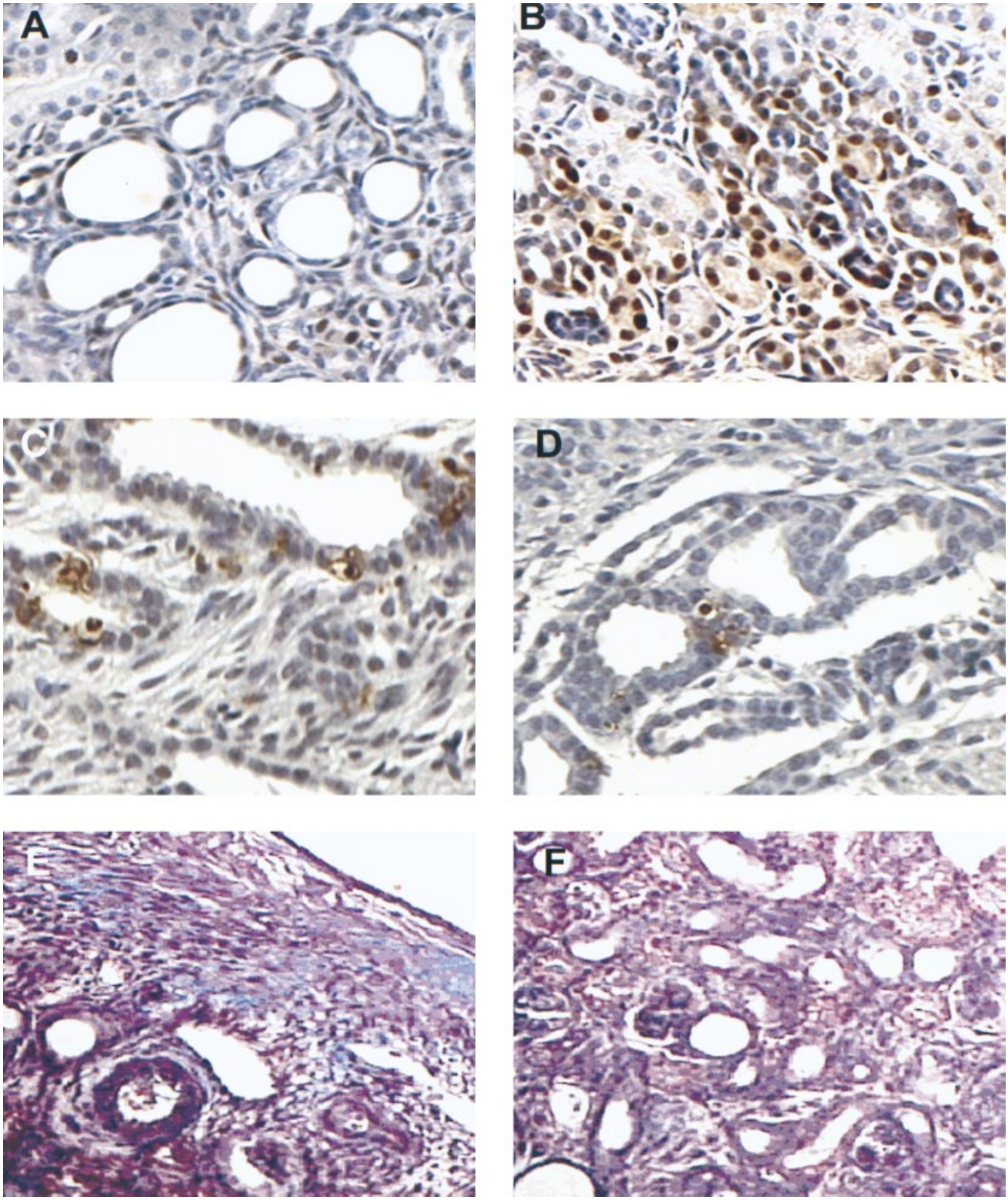

Fig. 1. Photomicrographs of representative obstructed (UUO) kidneys of neonatal rats. Control kidneys are shown on the left (A, C, E), and EGF-treated kidneys on the right (B, D, F). (*A and B*) Proliferating cell nuclear antigen (PCNA). (*C and D*) Apoptotic nuclei identified by Tdt uridine nick end label (TUNEL) technique. Labeling of cells in A-D is indicated by brown staining. (*E and F*) Interstitial collagen (blue) stained by Masson trichrome.

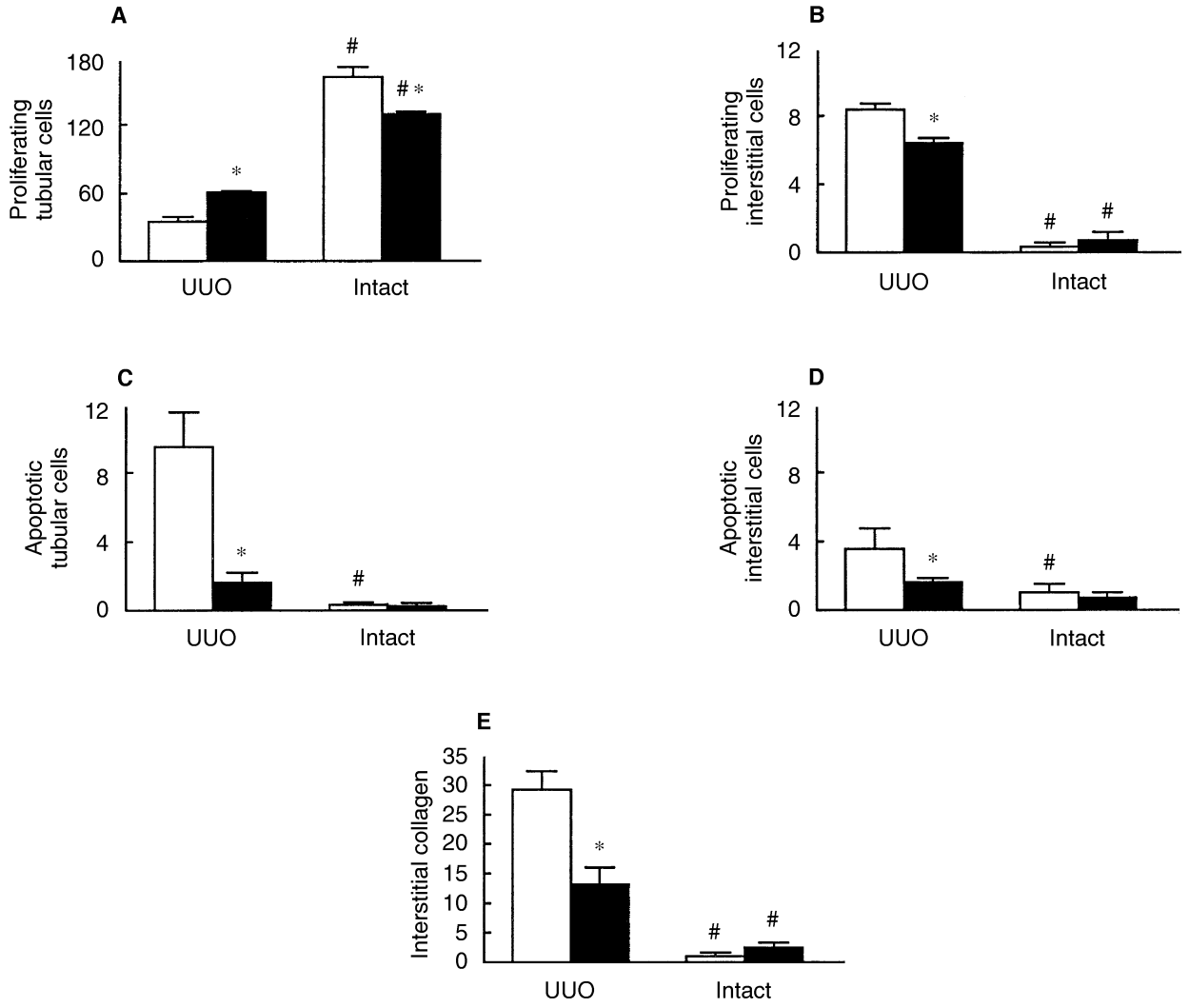

Fig. 2. Quantitation of cellular proliferation, apoptosis, and interstitial fibrosis in obstructed (UUO) and intact opposite kidney of control (□) and EGF-treated (■) groups of rats. (*A*) PCNA-labeled renal tubular epithelial cells ($N = 4$ each group). (*B*) PCNA-labeled interstitial cells (note different scale compared to panel A). (*C*) Tubular apoptotic cells identified by the TUNEL technique ($N = 4$ each group). (*D*) Interstitial apoptotic cells identified by TUNEL technique. (*E*) Relative area of renal interstitial collagen deposition ($N = 6$ each group). $^*P < 0.05$ versus control treatment group; $\#P < 0.05$ versus UUO kidney, same treatment group.

Figures 3F and 4F, compared to the intact kidney, UUO increased the number of atrophic tubules by tenfold ($P < 0.05$). The number of atrophic tubules in the obstructed kidney was reduced by 33% following administration of EGF (Fig. 4F).

Based on review of serial sections, proliferating (PCNA-labeled) or vimentin-staining tubular cells were distributed approximately equally between proximal tubules (binding Lotus tetragolonobus) and collecting ducts (binding Dolichos biflorus). In contrast, the majority of apoptotic or clusterin-staining tubular cells were localized to collecting ducts. The colocalization of proliferating and apoptotic cells in the obstructed kidney is shown in Figure 5.

DISCUSSION

The primary finding in the present study is the demonstration of a dramatic salutary effect of exogenous EGF on the cellular response of the developing kidney to chronic UUO. Whereas the approach to the pathophysiology of obstructive nephropathy had for many years focused on the hemodynamic consequences, the identification of a number of growth factors active in the kidney has more recently shifted attention to cellular events [7]. Thus, compared to the adult, the rapidly growing neonatal rat kidney appears to be particularly susceptible to interference with cellular proliferation and stimulation of apoptosis as a result of chronic UUO [6]. The mechanisms underlying these effects are complex, involving the interaction of multiple growth factors and cytokines.

The RTE cell appears to play a central role in the response to UUO. As shown in the present study, suppression of cellular proliferation and stimulation of apoptosis are primarily localized to the RTE cell. In response to UUO, RTE cells undergo phenotypic transformation to

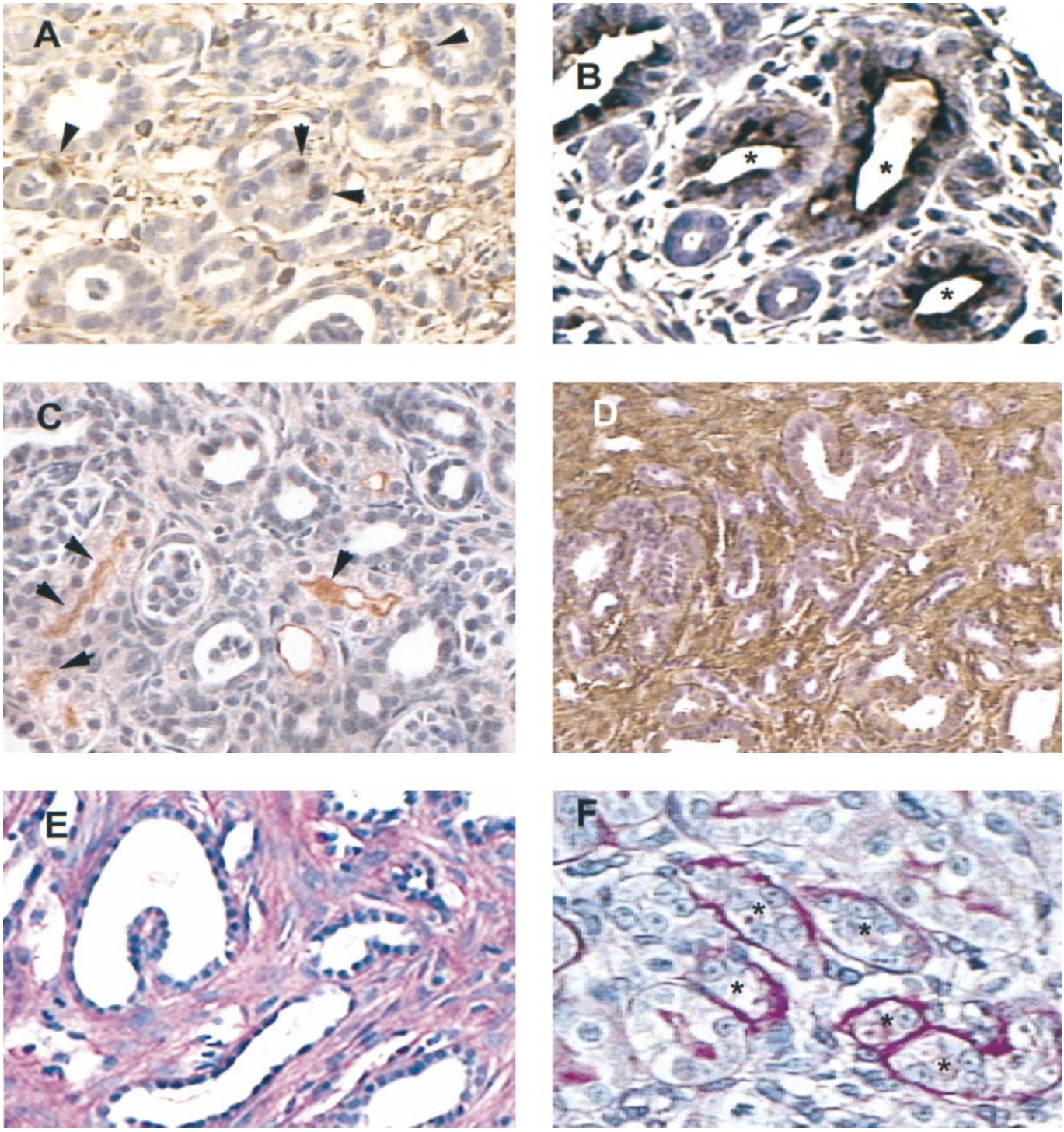

Fig. 3. Photomicrographs of representative obstructed (UUO) kidneys of control neonatal rats. (*A*) Immunoreactive vimentin distribution. Arrowheads indicate brown-staining vimentin-positive renal tubular epithelial (RTE) cells. (*B*) Immunoreactive clusterin distribution. Apical borders of RTE cells in dilated tubules contain brown-staining immunoreactive clusterin (*). (*C*) Immunoreactive transforming growth factor-beta 1 (TGF-β1) distribution. Arrowheads indicate brown-staining TGF-β1-positive tubules. (*D*) Immunoreactive alpha smooth muscle actin distribution. Interstitial cells are positively stained brown, whereas tubules are not. (*E*) Dilated tubules surrounded by pink-staining mesenchymal cells. (*F*) Atrophic tubules with thickened tubular basement membranes (*).

express vimentin, an intermediate filament protein produced by mesenchymal cells [16]. In preliminary studies, we have shown that expression of vimentin by RTE cells occurs following chronic obstruction of single nephrons by micropuncture [17]. In this regard, interstitial expression of alpha smooth muscle actin is also localized around dilated

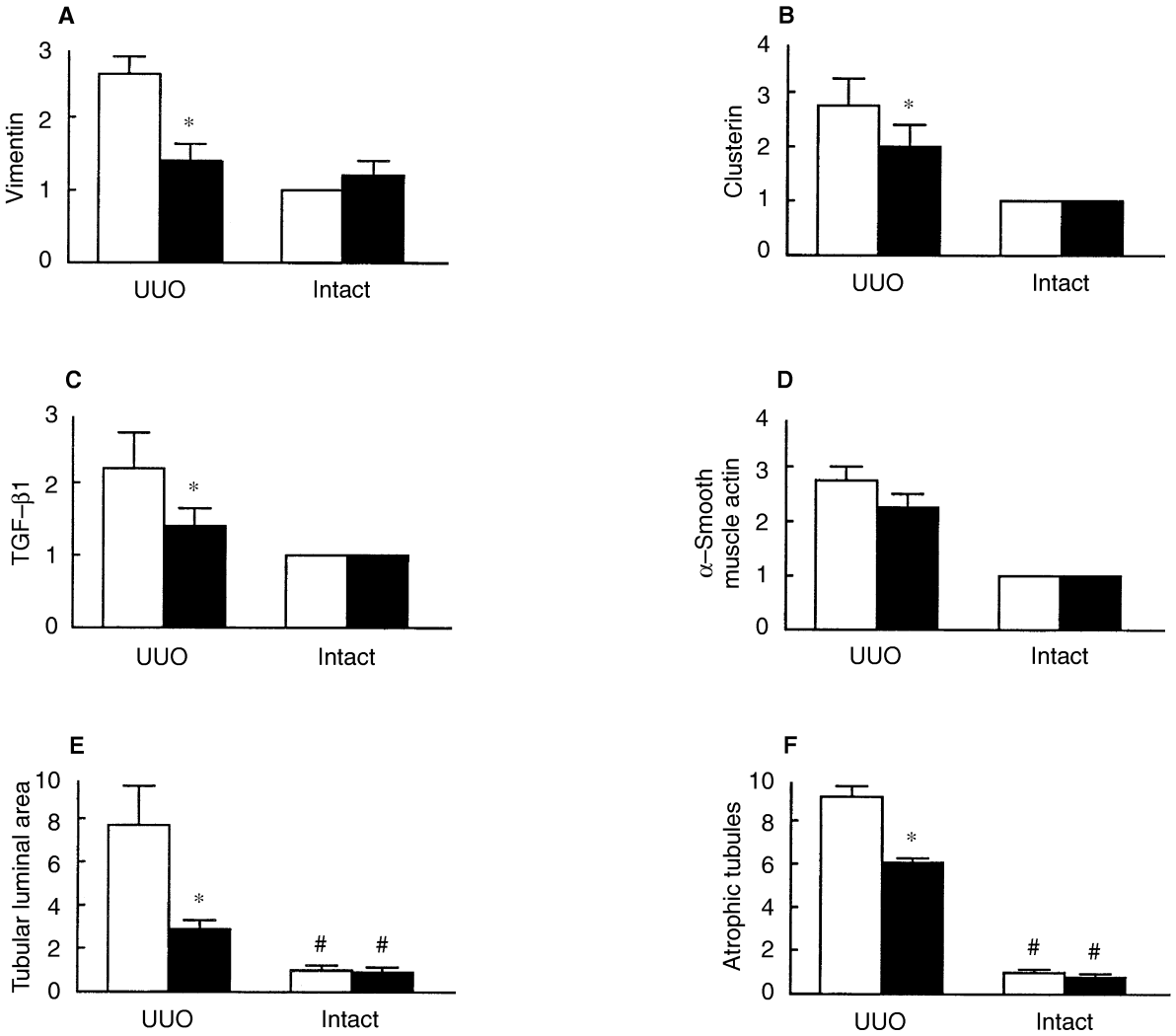

Fig. 4. Effect of epidermal growth factor (EGF) on indices of renal injury following UUO. (*A*) Semiquantitative distribution of immunoreactive vimentin in obstructed (UUO) kidney of each group. Shown is the relative number of vimentin-positive tubules per field ($N = 5$ rats each group). (*B*) Distribution of immunoreactive clusterin in obstructed kidney of each group. Shown is the relative number of clusterin-positive tubules per field ($N = 4$ rats each group). (*C*) Distribution of immunoreactive transforming growth factor-beta 1 (TGF-β1) in obstructed kidney of each group. Shown is the relative number of TGF-β1-positive tubules per field ($N = 5$ rats each group). (*D*) Distribution of immunoreactive alpha-smooth muscle actin in obstructed kidney of each group. Shown is the relative area of interstitial immunoreactive a-smooth muscle actin per field ($N = 4$ rats each group). (*E*) Relative mean tubular luminal area of each kidney of each group ($N = 6$ control, $N = 9$ EGF). (*F*) Relative number of atrophic tubules per field in each kidney ($N = 6$ per group). Shown are control (□) and EGF-treated (■) groups of rats;). $^*P < 0.05$ versus control treatment group; $\#P < 0.05$ versus UUO kidney, same treatment group.

loops of chronically obstructed single nephrons [17]. This suggests that the interstitial changes are also a consequence of alterations in neighboring RTE cells. Most importantly, the present study demonstrates that the renal consequences of chronic UUO in the neonate (tubular dilation, tubular atrophy, and interstitial fibrosis) are all attenuated by administration of EGF. Since EGF receptors are localized to RTE cells [18], this lends further support to the hypothesis that the RTE cell plays a critical role in the mediation of obstructive nephropathy. Chronic UUO reduced proliferation of RTE cells in the neonatal obstructed kidney. However, following EGF administration, RTE cell proliferation in the intact opposite kidney decreased in concert with an increase in cell proliferation in the obstructed kidney. This would appear to be an example of counterbalance, a concept originally developed by Hinman, in which alterations in one kidney are balanced by compensatory responses in the opposite organ [19]. Compared to the adult, counterbalance is highly activated in early development, including suppression of renin expression by the intact kidney while renin expression is markedly activated in the obstructed kidney [5]. By Northern analysis, EGF mRNA is undetectable in the neonatal rat kidney until three days of age [5], and renal immunoreactive EGF in the mouse has reached less than 50% of adult levels by two weeks of age [20]. Administration of EGF in a dose fivefold

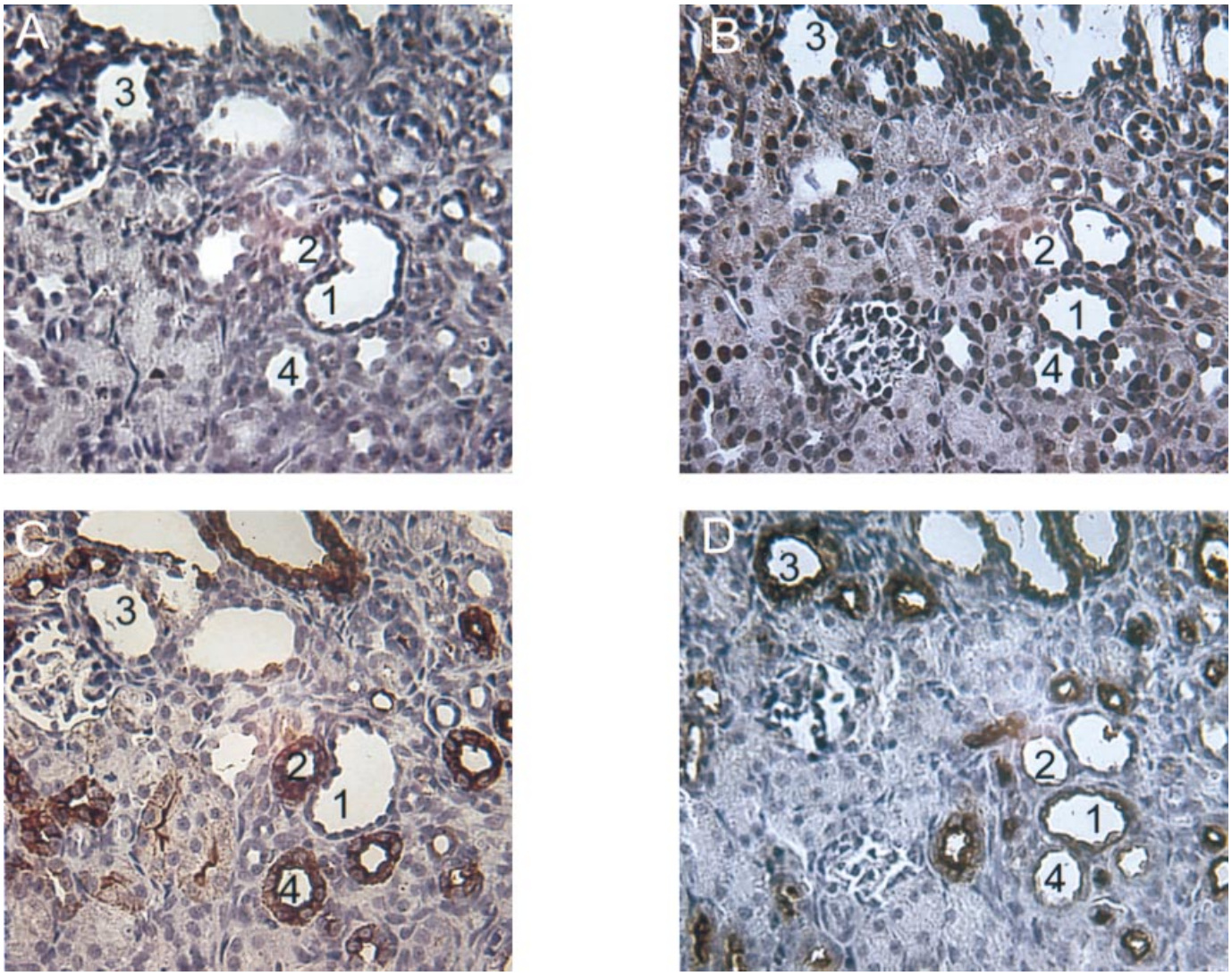

Fig. 5. Serial sections of obstructed kidney (UUO) from a saline-treated rat. The same four tubular loops have been identified in each section. (*A*) Apoptotic nuclei identified by the TUNEL technique. Tubules 1 and 3 contain positively stained cells. (*B*) Proliferating (PCNA-positive) cells are localized also to tubules 1, 2, 3, and 4. (*C*) Dolichos biflorus lectin, a marker for collecting ducts, binds to tubules 2 and 4. (*D*) Lotus tetragolonobus lectin, a marker for proximal tubules, binds to tubule 3.

that used in the present study impairs kidney growth in the newborn mouse [21]. It is possible that suppression of the renin-angiotensin system in the intact kidney contributes to a reduced effect of EGF on the intact opposite kidney.

In preliminary studies, we have shown that chronic UUO results in redistribution of EGF receptors from the basolateral to the apical surface of RTE cells [22]. Redistribution of EGF receptors to the apical cell surface has also been found in cystic kidney disease, in which the density of EGF receptors is also increased [23]. Thus, enhanced access of EGF to its receptor may also contribute to the response reported in the present study. In addition to its proliferative actions, EGF also stimulates cellular differentiation. Several lines of evidence suggest that tubular dilation associated with cystic kidney disease results from arrested differentiation of the RTE cell [24]. It is possible that clusterin expression by RTE cells in cystic kidneys is a consequence of dedifferentiation to a less mature stage [25]. In this regard, UUO in the neonatal rat results in the persistence of a number of markers of a less mature kidney, including the expression of clusterin [5]. Administration of EGF to RTE cells *in vitro* suppresses the expression of clusterin [26]. Thus, by stimulating its differentiation, exogenous EGF may allow normal development of the RTE cell, thereby reducing formation of tubular dilation in either the cystic kidney or the obstructed kidney. Chronic UUO stimulates greater clusterin expression in the adult compared to the neonatal obstructed kidney [6], and EGF reduces clusterin expression in RTE cells *in vitro* [26]. There are additional examples of congruence between cystic kidney disease and obstructive nephropathy. Elegant microdissection studies of chronic UUO in the fetal rabbit

demonstrate focal dilation of the distal nephron resulting in the formation of cysts, rather than dilation along the entire length of the nephron [27]. Also using techniques of microdissection, Tanner et al have shown recently focal regions of tubular stenosis in nephrons from rats with cystic kidney disease [28].

An additional action of EGF that may contribute to its salutary effects in the obstructed kidney is its promotion of RTE cell regeneration *in vitro* [29]. Thus, recovery of confluency by cultured RTE cells exposed to nephrotoxicants is markedly enhanced by exogenous EGF [30]. Others have shown that EGF promotes recovery of confluency of RTE cells following mechanical injury [29]. These findings suggest a role for EGF in accelerating the process of re-establishing a confluent, polarized epithelium by hastening the redifferentiation of RTE cells following transient dedifferentiation to cells with mesenchymal characteristics. The EGF-induced reduction in cellular expression of vimentin in the obstructed kidney in the present study is consistent with this hypothesis.

Growth and development are a net consequence of a balance between cellular proliferation and apoptosis. EGF has been shown to reduce apoptosis *in vitro*, as well as in other models of tubular injury [13, 14, 30]. EGF has been shown to inhibit apoptosis induced by transforming growth factor-β1 (TGF-β1) *in vitro* [31]. This occurs by inhibition of c-*fos* induction [31]. Since UUO in the neonatal rat markedly increases expression of renal TGF-β1 [5], it is likely that apoptosis is in part mediated by this cytokine, and that EGF may act in part through this mechanism. In addition to its action to inhibit apoptosis resulting from withdrawal of growth factors, EGF has been shown to oppose apoptosis resulting from oxidative injury in the kidney [32]. UUO has been shown to result in renal oxidative stress, which may be aggravated by insufficient response by renal antioxidant enzymes [33, 34]. Thus, EGF may reduce apoptosis at a point distal to the inciting stimulus. Administration of EGF to adult rats with 24 to 72 hours of UUO reduces apoptosis by 50% [10], which is comparable to the 80% reduction in apoptosis shown here in the neonatal rat. It is likely that apoptosis of RTE cells leads to tubular atrophy [35], in which case the effect of EGF to reduce apoptosis may account for the reduction in tubular atrophy in the present study.

In summary, chronic administration of EGF dramatically attenuated the impairment of renal development in the maturing rat kidney subjected to chronic UUO. RTE cell proliferation was augmented, apoptosis was suppressed, and RTE cell differentiation was restored. These cellular changes had profound long-term consequences, notably a reduction in tubular dilation, tubular atrophy, and interstitial fibrosis. Further investigation of these phenomena may elucidate the mechanisms underlying the regulation of abnormal renal growth and development, and should lead to new therapeutic approaches to congenital obstructive nephropathy.

ACKNOWLEDGMENTS

This research was supported in part by National Institutes of Health Research Center of Excellence in Pediatric Nephrology and Urology, DK44756 and DK52612; NIH O'Brien Center of Excellence in Nephrology and Urology, DK45179; and NIH Child Health Research Center, HD 28810.

Reprint requests to Robert L. Chevalier, M.D., Department of Pediatrics, Box 386, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908, USA.
E-mail: Rlc2 m@Virginia.Edu

APPENDIX

Abbreviations used in this article are: EGF, epidermal growth factor; PAS, periodic acid Schiff; PBS, phosphate buffered saline; PCNA, proliferating cell nuclear antigen; RTE, renal tubular epithelial cells; Tdt, terminal deoxynucleotidyl transferase; TGF, transforming growth factor; TUNEL, Tdt uridine-nick-end-label; UUO, unilateral ureteral obstruction.

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