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Research Article

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Water Deprivation Stimulates Transforming Growth Factor-β2 Accumulation in the Juxtaglomerular Apparatus of Mouse Kidney

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Abstract

Transforming growth factor-β (TGF-β) modulates the growth and differentiation of many cells and often functions in an autocrine or paracrine fashion. The myoepithelial cells of the renal juxtaglomerular apparatus (JGA) synthesize and secrete renin. Under conditions which chronically stimulate renin production, the JGA undergoes hypertrophy and hyperplasia. The molecular factors responsible for these changes in the JGA have not been identified. In the present study, plasma renin activity was stimulated in the mouse by water deprivation. Using immunoperoxidase staining with specific antibodies against TGF-β1, β2, and β3, we found increased TGF-β2 accumulation in the JGA and interlobular arteries. Immunostaining with renin antiserum demonstrated colocalization of TGF-β2 and renin. TGF-β1 and β3 expression was not different between control and water-deprived mice. Our results suggest that in the setting of water deprivation, TGF-β2 is localized in a manner which would allow it to act either as a growth factor for or as a phenotypic modulator of the JGA and renal arterioles. (*J. Clin. Invest.* 1991. 88:2117–2122.) Key words: transforming growth factor-β • juxtaglomerular apparatus hyperplasia • arterial smooth muscle • renin • angiotensin II

Introduction

Transforming growth factor-βs (TGF-β)[1] are multifunctional polypeptides which have complex effects in development, cell proliferation, and differentiation (1). In general, TGF-βs promote differentiation in many cell types. While they inhibit proliferation in most cells (e.g., fibroblasts, renal epithelial cells, lymphocytes), they promote proliferation in certain cells (e.g., osteoblasts). TGF-βs are present in renal cortex and medulla (2–4) and have recently been implicated in the enhanced synthesis of extracellular matrix components in experimental glomerular disease (5, 6).

The juxtaglomerular apparatus (JGA) of the mammalian kidney is composed of three cell types: specialized epithelial cells of the distal renal tubule which constitute the macula densa, the Goormaghtigh cells of the extraglomerular mesangium, and the myoepithelial granular cells of the glomerular arteriole which secrete both renin and angiotensin II (7). Several chronic stimuli (including renal ischemia, dietary electrolyte depletion, extracellular fluid volume contraction, and pharmacologic inhibition of angiotensin-converting enzyme) increase plasma renin activity and, in some cases, induce hypergranulation and hyperplasia of the JGA and the afferent glomerular arteriole. The molecular mechanisms responsible for this hypergranulation and hyperplasia are unknown.

Using an immunohistochemical approach, we have examined the expression of TGF-βs in the JGA using specific antibodies prepared against synthetic peptides derived from the primary sequences of TGF-β1, β2, and β3. We selected as a model severe dehydration induced by water deprivation in the mouse, which induces dehydration and renal sodium wasting (8), as well as elevated plasma renin activity. We found that water deprivation is associated with a selective increase in TGF-β2 accumulation in the JGA and small renal arteries, localized to cells which also accumulate immunoreactive renin.

Methods

Experimental design. 6-wk-old female FVB/N mice (Harlan Sprague Dawley, Indianapolis, IN), weighing 20±1 g were fed a standard laboratory mouse diet (ICN Biochemical Co., Cleveland, OH). The control group (n = 25) was given water ad libitum, whereas a second group (n = 25) underwent water deprivation for 72 h. At the end of this period, animals in both groups were sacrificed by decapitation. A third group (n = 25) was sacrificed after 3 d of water deprivation followed by 24 h of rehydration. Body weight was measured at the beginning and at the end of each experiment. Blood was obtained from each animal by retroorbital puncture, total serum protein concentration was measured by an AO refractometer (Leica, Deerfield, IL) and hematocrit was measured by centrifugation in a capillary tube. Using blood obtained after decapitation and pooled from all animals in each experimental group, serum sodium concentration was measured with a KNA2 sodium-potassium analyzer (Radiometer, Copenhagen, Denmark). Using pooled blood obtained after decapitation and collected in ice-cold tubes containing EDTA, renin activity was measured by radioimmunoassay (9). Data are presented as mean±SD.

Antibodies. Affinity-purified rabbit polyclonal antibodies directed against peptide sequences unique to TGF-β1, β2, and β3 were prepared and have been shown previously to have isoform specificity (10–13). The peptide sequences were from the latency-associated peptide of the TGF-β1 precursor, and from the mature forms of TGF-β2 and TGF-β3. Polyclonal rabbit antiserum prepared against mouse salivary gland renin was kindly provided by Dr. Nakayama and Dr. Murakami, University of Tsukuba, Tsukuba, Ibaraki, Japan.

Immunohistochemical staining. Kidneys were fixed in zinc formalin (Anatech, Battle Creek, MI) and embedded in paraffin. TGF-β isotypes were localized in 5-μm tissue sections using an avidin-biotin-peroxidase kit (Vector Laboratories, Inc., Burlingame, CA). Deparaf-

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1. *Abbreviations used in this paper:* JGA, juxtaglomerular apparatus; TGF-β, transforming growth factor-beta.

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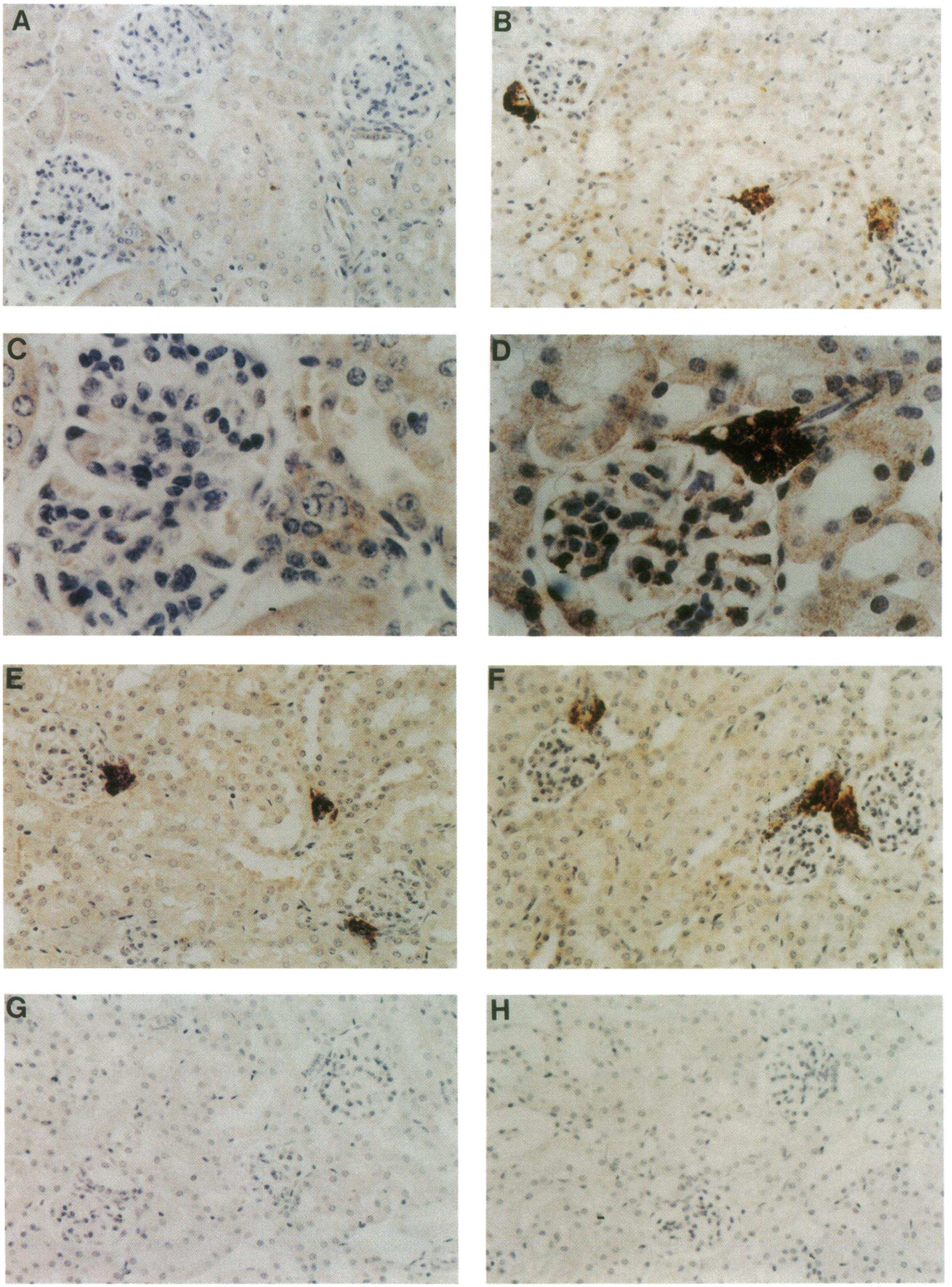
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Table I. Systemic Effects of Dehydration in Mice

	Serum [Na]	Total protein	Hematocrit	Basal weight	Final weight	Plasma renin activity
	mEq/liter	*g/dl*	*%*	*g*	*g*	*ng/ml per h*
Control	144±1.7	5.6±0.2	45±1.2	20.1±0.3	20.1±1.6	2.7
Dehydration	159±2.3	6.5±0.2	50±1.3	20.0±0.9	15.6±0.9	7.1

Mice were provided water ad libitum (control) or denied water (dehydration) for 3 d. Mean±SD are presented for groups with the following sizes: serum sodium, total protein, and hematocrit, six mice; basal and final weight, five mice; and plasma renin activity, 10 control mice and 18 dehydrated mice.

finized sections were digested with hyaluronidase (1 mg/ml, Calbiochem-Behring Corp., La Jolla, CA), blocked with 5% normal goat serum, 1% BSA fraction V (Miles Laboratories Inc., Naperville, IL), and 1% ovalbumin (Fluka, Inc., Ronkonkoma, NY), and incubated with primary antibodies (4–10 μg/ml for anti-TGF-β antibodies, 1:6,000 dilution for antirenin antiserum) in 1% BSA in Tris-buffered saline (TBS) for 16 h at 4°C. The sections were washed in TBS/0.1% BSA, incubated with biotinylated goat anti-rabbit IgG, washed and incubated with avidin-biotin complex. The sections were incubated in 3,3'-diaminobenzidine (Sigma Chemical Co., St. Louis, MO) (0.5 mg/ml) in 0.1% hydrogen peroxide for 3–5 min and counterstained with Mayer's hematoxylin. Controls included replacing the primary antibody with equivalent concentration of nonimmune rabbit IgG and using primary antibody that had been blocked by preincubation with a 20-fold molar excess of the immunizing peptide.

Results

During 3 d of water deprivation, mice lost 22% of their basal weight, from 20.0±0.9 g to 15.6±0.9 g (Table I). The difference in serum sodium concentration between control mice (144 mEq/liter) and dehydrated mice (159 mEq/liter) indicates severe water depletion. The difference in hematocrit (45±1.2% in control mice and 50±1.3% in dehydrated mice) suggests a component of extracellular volume contraction as well. Dehydrated mice given free access to water for 24 h regained 65% of the lost weight (initial weight 20.1±0.3 g, dehydrated weight 15.3±1.0 g, rehydrated weight 18.4±1.0 g). Plasma renin activity was 2.7 ng/ml per h in a pooled specimen derived from 10 control animals and 7.1 ng/ml per h in a pooled specimen derived from 18 dehydrated animals.

Faint staining of tubular epithelial cells and glomerular cells was observed in control mouse kidney using the TGF-β2 antibody (Fig. 1 *A*). After 3 d of water deprivation, intense staining of TGF-β2 was noted within the JGA (Fig. 1 *B*). Higher magnification views of glomeruli from control mice (Fig. 1 *C*) and water-deprived mice (Fig. 1 *D*) confirm the localization of TGF-β2 to the JGA. TGF-β2 staining in other portions of the kidney, including the tubular epithelial cells, was unaffected by water deprivation. The intense staining for TGF-β2 in JGA did not diminish after 24 h of rehydration (data not shown). Immunoreactive renin was localized to the JGA of control mouse kidney (Fig. 1 *E*). With water deprivation renin staining was noted within both the JGA and interlobular arteries (Fig. 1 *F* and Fig. 2 *A*). In contrast, nonimmune rabbit IgG did not stain the sections (Fig. 1 *G*) and anti-TGF-β2 antibody preincubated with TGF-β2 peptide demonstrated greatly diminished staining (Fig. 1 *H*). TGF-β2 peptide had no effect on staining by TGF-β3 antibody, excluding a nonspecific inhibition by this peptide (data not shown).

In dehydrated mice, renin antiserum stained JGA and small renal arteries which appeared by their diameter to be interlobular arteries (Fig. 2 *A*). Immunoreactive TGF-β2 was found in the same distribution, and localized to the smooth muscle layer of the interlobular artery (Fig. 2 *B*).

In both control and dehydrated kidneys, diffuse staining of tubular epithelial cells was seen with TGF-β1 antibody (Fig. 3, *A* and *B*), whereas certain cortical tubules were stained intensely with TGF-β3 antibody (Fig. 3, *C* and *D*). No differences in staining with antibodies to TGF-β1 and β3 were apparent after water deprivation.

Discussion

In the present study, immunoreactive TGF-β2 was localized predominantly to renal tubular epithelial cells in control mice. After water deprivation lasting 3 d, TGF-β2 accumulation was markedly increased in the JGA and interlobular arteries, colocalizing with immunoreactive renin. Due to the limitations inherent in immunohistochemical technique, it cannot be ascertained whether the increased intracellular accumulation of TGF-β2 in the setting of dehydration is due to increased synthesis, retarded secretion, or increased uptake of extracellular TGF-β2. TGF-β1 and β3 were localized primarily to renal

Figure 1. TGF-β2 and renin localization in mouse kidney. Control mouse kidney demonstrates faint staining for TGF-β2 in tubular epithelial cells and glomeruli (*A*, original magnification, 320). Water-deprived mouse kidney demonstrates similar staining to control in the tubular epithelial cells and glomeruli, together with greatly enhanced TGF-β2 staining in the JGA (*B*, magnification, 320). Higher magnification views of glomeruli from a control mouse (*C*, magnification, 800) and water-deprived mouse (*D*, magnification, 800) confirm localization of immunoreactive TGF-β2 within the JGA of a water-deprived mouse. Immunoreactive renin is localized to the JGA in a control mouse (*E*, magnification, 320) and a water-deprived mouse (*F*, magnification, 320). Staining is absent from the JGA of water-deprived mouse kidney using nonimmune serum IgG (*G*, magnification, 320) and is greatly diminished when anti-TGF-β2 antibody was preincubated with excess immunizing peptide (*H*, magnification, 320).

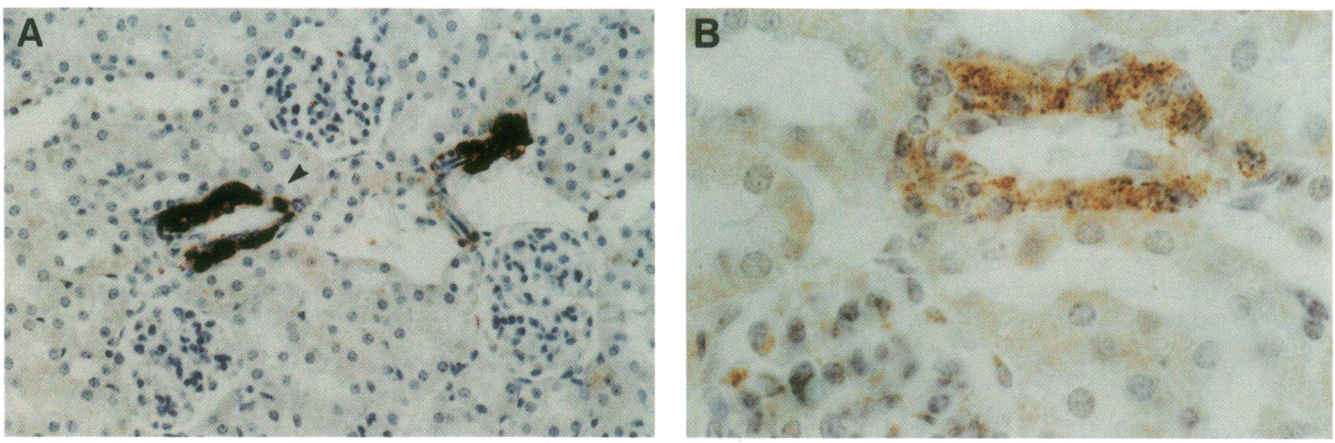

Figure 2. Renin and TGF-β2 localization in renal arteries. In a water-deprived mouse (*A*, magnification, 320), renin staining is present in an interlobular artery (*arrowhead*). Immunoreactive TGF-β2 is also expressed in the interlobular artery, predominantly within the smooth muscle layer (*B*, magnification, 800).

tubular epithelial cells and the intensity of staining was unaffected by water deprivation.

The pattern of distribution of TGF-β2 within the kidney of dehydrated mice is strikingly similar to that of renin. It is unlikely that the colocalization of TGF-β2 and renin is due to cross-reactivity of the antibodies employed, for two reasons. First, the anti-TGF-β2 antibodies used were raised against peptide sequences, and there is no sequence homology between TGF-β2 and renin. Second, the JGA of control mice stained prominently with the anti–renin antibody but not with the

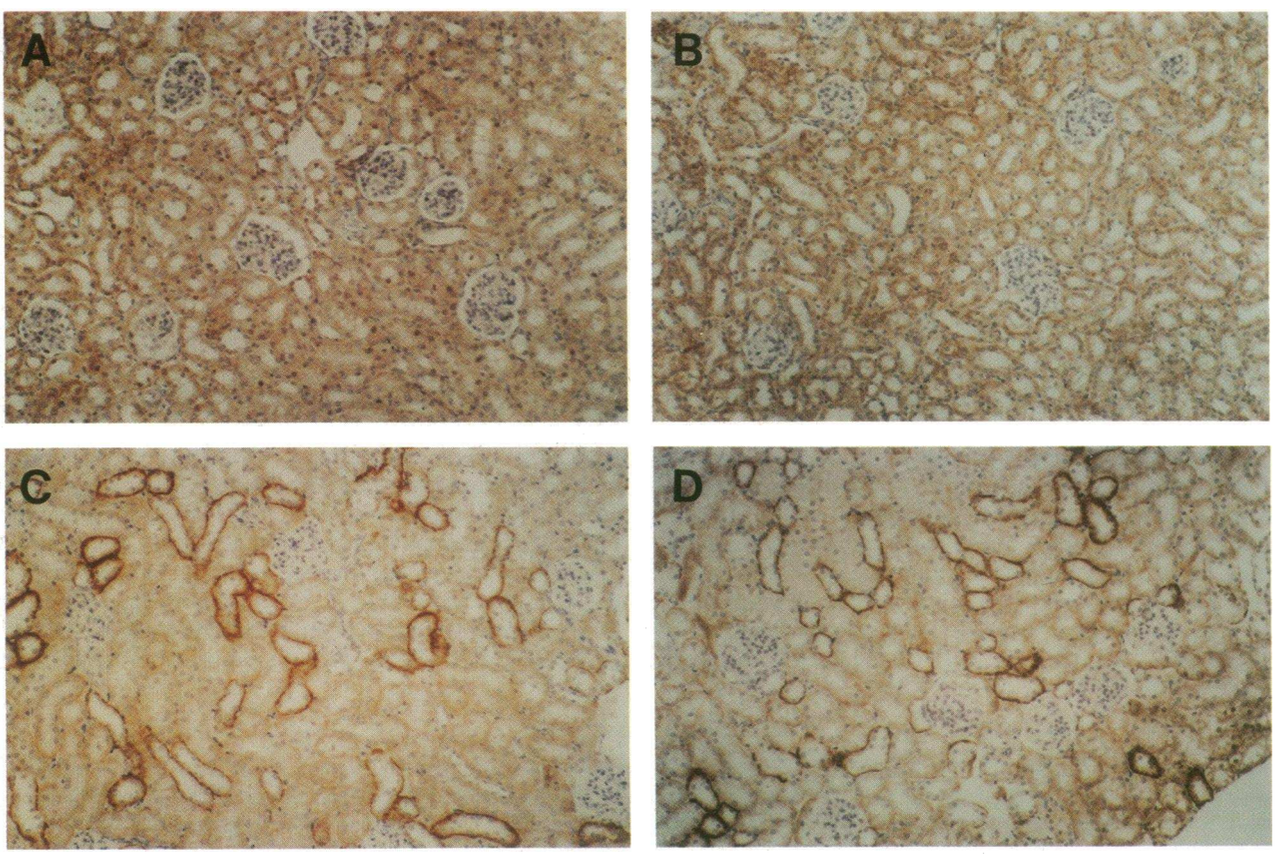

Figure 3. TGF-β1 and β3 expression in mouse kidney. Immunoreactive TGF-β1 is localized to tubular epithelial cells of a control (*A*, magnification, 160) and water-deprived mouse (*B*, magnification, 160) with similar distribution and intensity of staining. Immunoreactive TGF-β3 is found in the renal tubular epithelial cells of a control (*C*, magnification, 160) and water-deprived mouse (*D*, magnification, 160) with similar distribution and intensity of staining.

anti-TGF-β2 antibody. In the adult rat, renin is localized to the JGA, and after stimulation, renin expression extends proximally to the interlobular renal arteries (7). The data presented here indicate that after water deprivation, TGF-β2 has an identical distribution, localizing to the JGA and small renal arteries. Direct demonstration that renin-secreting cells also transcribe TGF-β2 mRNA and synthesize the protein will require colocalization of renin and TGF-β2 mRNA by in situ hybridization and colocalization of these proteins by immunoelectron microscopy.

TGF-βs constitute a family of multifunctional growth factors, whose activities depend upon the target cell, the degree of cellular differentiation, and the cellular environment, including the presence of other growth factors (1). Whereas TGF-β1 and β2 share many activities, TGF-β2 has a unique role in development, particularly in the process of mesodermal induction (14, 15). Cultured cell lines secrete TGF-β1 and β2 in different proportions, which further suggests that these isoforms are differentially regulated (16). In the present study, water deprivation was associated with an increase in one isoform, TGF-β2, which was limited to the JGA and small renal arteries. The precise spatial distribution of TGF-β2 suggests that this isoform may have a distinct functional role in kidney.

TGF-βs are known to regulate the phenotype of certain cultured smooth muscle cells. Thus, TGF-βs inhibit DNA synthesis and attenuate the effects of many mitogenic polypeptides on cultured renal glomerular mesangial cells, although this effect may depend, in part, on cell density (3, 17). Similarly, TGF-β acts on cultured aortic smooth muscle cells to inhibit proliferation and increase cell size and protein content (18). Another local peptide factor, angiotensin II, is mitogenic for human aortic smooth muscle cells (19) and human glomerular mesangial cells (20). By contrast, angiotensin II is not mitogenic for cultured rat aortic smooth muscle cells but increases cell size and protein content (21–23). The recent demonstration that angiotensin II increases steady-state levels of TGF-β mRNA in cultured rat aortic smooth muscle cells raises the possibility that TGF-β may mediate the hypertrophic effect of angiotensin II (24). Thus during dehydration, stimulation of the renin axis, and in particular of angiotensin II, may be responsible for the increased TGF-β2 expression by smooth muscle cells of the renal arteries and by myoepithelial cells of the JGA.

An attractive model to explain hypertrophy and hyperplasia of the JGA in states associated with increased renin production suggests that angiotensin II stimulates renal TGF-β expression and that TGF-β acts in a paracrine or autocrine fashion to promote cellular hypertrophy. On the other hand, angiotensin-converting enzyme inhibition is also associated with hypertrophy and hyperplasia of the JGA (25). This would suggest that renin itself, rather than angiotensin II, might stimulate TGF-β2 synthesis. Alternatively, other factors might stimulate both renal TGF-β2 and JGA hypertrophy in this setting.

In addition to promoting cellular hypertrophy, TGF-βs might also modulate renin synthesis by renal arteries and the JGA. In several systems, TGF-βs regulate hormone synthesis and secretion (26, 27). Of particular relevance to the present study is the observation that TGF-β inhibits steroidogenesis and decreases angiotensin II receptor number in adrenal cortical cells (28). Further investigation should examine whether TGF-β2 modulates renin synthesis by cultured juxtaglomerular cells, and whether it modulates angiotensin II receptor number by renal vascular smooth muscle or mesangial cells.

In conclusion, we have demonstrated that water deprivation in the mouse is associated with enhanced accumulation of TGF-β2 in the kidney juxtaglomerular cells and in small renal arteries. Our results suggest that TGF-β2 may act as a local mediator of the hyperplastic and hypertrophic response of the JGA in states associated with increased renin production.

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