Hepatocyte growth factor induces an endothelin-mediated decline in glomerular filtration rate

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Biswas, Purba, Abinash Roy, Rujun Gong, Angelito Yango, Evelyn Tolbert, Jason Centracchio, and Lance D. Dworkin. Hepatocyte growth factor induces an endothelin-mediated decline in glomerular filtration rate. Am J Physiol Renal Physiol 288: F8–F15, 2005; doi:10.1152/ajprenal.00435.2003.—Hepatocyte growth factor (HGF) is a multifunctional cytokine that plays a crucial role in renal development, injury, and repair. HGF also serves a protective role in chronic renal disease by preventing tissue fibrosis. Endothelin-1 (ET-1), produced primarily by endothelial cells, is a potent vasoconstrictor that also acts as a proinflammatory peptide, promoting vascular injury and renal damage. In addition to mediating a variety of epithelial cell responses, HGF also induces hemodynamic changes that are poorly understood. The aim of the present study was to study the acute and chronic effects of HGF on ET-1 production in the kidney. We hypothesized that hemodynamic changes upon HGF treatment are likely mediated by immediate ET-1 release, whereas protection from renal fibrosis in rats chronically treated with HGF is likely due to suppression of ET-1 production. Acute HGF infusion into rats caused a decline in blood pressure that was enhanced by pretreatment with bosentan (an endothelin A and B receptor antagonist). HGF infusion also resulted in a decline in glomerular filtration rate (GFR) that could be entirely prevented by bosentan, suggesting that HGF acutely increases production and/or release of ET-1, which then mediates the observed decline in GFR. In cultured glomerular endothelial cells, HGF induced ET-1 production in a dose-dependent manner. Moreover, although there was an initial increase in ET-1 production upon HGF treatment, longer administration suppressed ET-1 production. This finding was consistent with the observation in vivo of a decrease in ET-1 production in renal parenchyma of rats chronically treated with HGF. Our data suggest both a hemodynamic and biological role for HGF-mediated ET-1 regulation.

In the kidney, HGF ameliorates chronic injury in a variety of settings, including the remnant kidney model (9, 12), unilateral ureteral obstruction (44), and diabetic nephropathy (30). Treatment with HGF either at the onset or after disease is established attenuates renal fibrosis (9, 12, 19, 44). HGF prevents apoptosis of tubular epithelial cells (26), blunts epithelial-to-mesenchymal transdifferentiation (22, 41, 43), and, as we have shown, inhibits matrix accumulation by activating matrix metalloproteinase and plasminogen activator/plasmin matrix proteolytic pathways (9, 12, 25). However, the exact mechanism(s) by which HGF retards injury is still uncertain. HGF has also been demonstrated to produce an acute reduction in mean arterial pressure (MAP) and increase in heart rate, presumably through venodilation (35). The physiological role of HGF in the control of the systemic and renal vasculature is not well studied and, in particular, whether HGF has significant effects on renal hemodynamics is unknown.

Endothelin-1 (ET-1) is a 21-amino acid peptide that is a potent vasoconstrictor and vascular growth factor. Increased ET-1 production has been associated with several renal disorders, such as transplant rejection, disease progression in the remnant kidney model, diabetic nephropathy, and acute renal failure. ET-1 acts on ETA and ETB receptors, is involved in the pathogenesis of acute and chronic renal failure, and mediates the nephrotropic effects of cyclosporine A and tacrolimus. ETA receptors are abundant in vascular smooth muscle cells and mediate the pressor response to ET-1 (18), whereas ETB receptors are present on endothelial cells and mediate endothelium-dependent vasodilatation via nitric oxide (NO) formation (10). ET-1 is produced in the kidney, and both ETA and ETB receptors are also distributed in the kidney (33). All physiological glomerular actions of ET-1 are mediated by the ETB receptors (35). The stimulatory effect of cyclosporine A and tacrolimus on ET-1 release from cultured proximal tubule epithelial cells is antagonized by HGF and EGF (14, 15). Prolonged treatment of endothelial cells with HGF decreased ET-1 production (15, 17).

ANG II, the key effector of the local and circulating renin-angiotensin system, plays a central role in blood pressure regulation. ANG II is a potent vasoconstrictor and also stimulates ET-1 synthesis and release in vascular smooth muscle and endothelial cells. Although several reports have demonstrated the role of ANG II inhibition in increasing HGF levels, the role of HGF in modulating ANG II release and effects has hardly been studied.

HEPATOCTYE GROWTH FACTOR (HGF) is a mesenchymally derived (38), pleiotropic (29), multifunctional growth factor (32) that acts as an important regulator of diverse processes, including mitogenesis (11), motogenesis (11), morphogenesis (31), and apoptosis (21), in a variety of cell types. The HGF receptor is c-met (6, 39), a transmembrane tyrosine kinase prominently expressed by epithelia (13). C-met is also expressed in vascular tissue, including endothelial and vascular smooth muscle cells (37). In vivo, HGF is essential for embryogenesis (4) and tumorigenesis (8) and is implicated in organ repair after injury (5). After acute organ damage, circulating HGF levels rise and responses are targeted by site-specific induction of c-met (23, 36, 40, 42).
The purpose of the present study was to examine the acute and chronic effects of HGF on systemic and renal hemodynamics and glomerular filtration rate (GFR) in the normal rat. In addition, we investigated the roles of ET-1 and ANG II in the genesis of the HGF-induced vascular response by using specific antagonists for these substances. Because we found that HGF had significant hemodynamic effects that could be blocked by an ET-1 receptor antagonist, we also explored the cellular actions of HGF on ET-1 production in glomerular endothelial cells.

**METHODS**

Studies were performed in male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 170-270 g. Rats were fed standard rat chow and had free access to water. The institution’s Animal Use and Care Committee approved all studies.

*Design of the acute study.* Rats were fasted overnight but had free access to water. On the day of study, rats were anesthetized with thiobutabarbital (100 mg/kg ip; Andrew Lockwood, Sturtevant, WI) and placed on a heated table to maintain constant body temperature. A polyethylene catheter (PE-50) was inserted in the femoral artery, and mean arterial pressure (MAP) was continuously monitored by a pressure transducer connected to a personal computer. The arterial catheter was also used to obtain blood samples for determination of hematocrit and inulin concentration. A tracheotomy was performed, and additional catheters were inserted in the jugular veins for infusion of solutions. The left kidney and the ureter were exposed by a left subcostal incision, and a catheter was inserted into the ureter for urine collection. A 6-mm section of left renal artery was dissected free, with care taken not to damage the renal nerves, and a 1-mm probe connected to an ultrasonic blood flowmeter (model T106XM, Transonic Systems, Ithaca, NY) was placed on the renal artery to monitor renal blood flow (RBF).

To compensate for losses, all rats received an initial volume of rat plasma of 10 ml/kg body wt followed by a sustained intravenous (iv) infusion of plasma at 0.5 ml/h adjusted to maintain a stable hematocrit. Rats also received a 0.5 ml iv bolus of [3H]methoxyinulin followed by a sustained infusion at a rate of 0.5 ml/h. After a 45-min equilibration period, two 15-min urine samples were collected with midpoint blood samples. MAP and RBF were recorded at the time of blood sample collection. At the end of the baseline period, rats received an iv bolus or infusion (see below) of a test substance or vehicle. After a 30-min equilibration period, two more sets of urine and blood samples were collected and MAP and RBF were again recorded.

Urine was collected in preweighed tubes, and urine volume was determined gravimetrically. Urine and plasma inulin content were determined by liquid scintillation counting, and GFR and renal plasma flow (RPF) were calculated by the standard formulas (23).

Six groups were studied (see Table 1). Rats received either no pretreatment, the ET receptor blocker bosentan (200 mg/kg) by gavage 1 h before the study, or the ANG II receptor antagonist candesartan (5 mg/kg), which was added to their drinking water for 24 h before the study (Astra/Zeneca). After the baseline period, rats received vehicle, ET-1, or an intravenous bolus of HGF (20 μg/kg, Genentech, South San Francisco, CA) in 0.1 ml saline.

*Design of the chronic study.* All animals underwent sterile, subcutaneous implantation of an osmotic minipump (Alzet Micro-Osmotic Pump, Alza, Palo Alto, CA) that delivered HGF or saline intravenously into the jugular vein for 1 wk. Briefly, rats were anesthetized with 50 mg/kg of ketamine intraperitoneally (ip) and 0.5 mg/kg of metomidine intramuscularly. A small incision was made in the neck area, and a pocket was created to accept the pump. The left jugular vein was exposed and cannulated with polyethylene tubing connected to the minipump. The incision was closed with sterile staples, and 1.0 mg/kg of ip atipamezole was given for anesthetic recovery. Rats were observed until fully awake.

Animals were divided into two groups. Control rats (CON; n = 7) received normal saline, and HGF rats (n = 6) received 100 μg·kg HGF⁻¹·day⁻¹.

The rats were maintained on standard rat chow and water ad libitum for 6 days. On day 7, rats were anesthetized, and MAP, GFR, and RPF were assessed as described above.

*Determination of plasma and urine HGF levels by ELISA.* The plasma concentration of HGF was measured using a specific sandwich ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. For the acute study, HGF concentration was determined in the plasma of two rats that received HGF and in one control rat at 0, 15, 30, 60, 120, 240, and 360 min after the iv bolus of vehicle or HGF. Urine HGF was measured in three HGF and one CON rats at 0, 15, 30, 60, 120, and 240 min after the bolus of HGF. For the chronic study, plasma HGF concentration was measured in three CON and four HGF-treated rats on days 2, 5, and 7 after implantation of the pump. Serum and urine HGF levels were measured in the same three HGF-treated rats. The rationale for measuring urine and serum HGF levels after HGF infusion was to verify that we would indeed achieving higher than physiologically circulating levels of HGF in these animals.

*Immunostaining for ET-1 in kidneys of rats infused with HGF.* To examine the effects of chronic HGF infusion on renal ET-1 production in vivo, kidneys of rats in the chronic study were perfusion fixed with formalin and embedded in paraffin, and 3-μm sections were prepared. Immunoperoxidase staining was performed with a Vectastain ABC kit (Vector Laboratories, Burlingame, CA). Briefly, sections were deparaffinized, hydrated, and blocked with normal goat serum overnight, then incubated with a rabbit polyclonal endothelin antibody (1:1,000; Oncogene, Cambridge, MA) for 1 h. Sections were incubated with biotinylated goat anti-rabbit IgG secondary antibody for 30 min and subsequently detected using the avidin-biotin-peroxidase system. Nonimmune rabbit serum was used as a negative control. Tissue expression of ET-1 in both glomeruli and tubulointerstitium was graded on a scale from 0 to 3: 0, absent; 1, mild; 2, moderate; and 3, severe. A mean score was calculated using the values obtained in 20–30 glomeruli or 20 random high-power (×400) fields/rat in 3 rats/group.

*Bovine glomerular endothelial cell culture and ET-1 assay.* Bovine glomerular endothelial cells (BGEI Vec Technologies, Lake Placid, NY) were grown on 0.2% gelatin-coated plates in MDCB medium (Vec Technologies). Cells (2 × 10⁴ cells) were plated into 24-well plates and grown to confluence. They were starved for 12 h in 0.1% FBS containing DMEM, followed by addition of 100 ng/ml recombinant HGF in serum-free DMEM for the various time points studied. For the HGF time course, quiescent BGE were treated with 1, 10, and 100 ng/ml of HGF in serum-free DMEM for the various time points studied. The rationale for measuring urine and serum HGF levels after HGF infusion was to verify that we indeed achieving higher than physiologically circulating levels of HGF in these animals.

### Table 1. Summary of groups in the acute study

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Pretreatment</th>
<th>Substance Given After Baseline Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>12</td>
<td>None</td>
<td>Vehicle</td>
</tr>
<tr>
<td>BOS-CON</td>
<td>7</td>
<td>Bosentan (200 mg/kg gavage)</td>
<td>Vehicle</td>
</tr>
<tr>
<td>BOS-ET</td>
<td>8</td>
<td>Bosentan (200 mg/kg gavage)</td>
<td>Endothelin-1 (50 μg/kg · min⁻¹ · iv)</td>
</tr>
<tr>
<td>HGF</td>
<td>12</td>
<td>None</td>
<td>HGF (20 μg/kg iv bolus)</td>
</tr>
<tr>
<td>BOS-HGF</td>
<td>10</td>
<td>Bosentan (200 mg/kg gavage)</td>
<td>HGF (20 μg/kg iv bolus)</td>
</tr>
<tr>
<td>CAN-HGF</td>
<td>11</td>
<td>Candesartan (5 mg/kg gavage)</td>
<td>HGF (20 μg/kg iv bolus)</td>
</tr>
</tbody>
</table>

Summary of nos. (n) of animals/group and treatments applied to the various groups of rats is shown. CON, control; BOS, bosentan; ET, endothelin-1; HGF, hepatocyte growth factor; CAN, candesartan.
100 ng/ml of HGF for 30 min. Supernatants were collected and centrifuged at 14,000 rpm at 4°C for 10 min, and 100 μl were used for ELISA using a commercially available kit from R&D Systems. Data are representative of four independent experiments.

Statistical analysis. Statistical analysis was performed on a personal computer running Sigmasstat statistical software (Jandel Scientific Software, San Rafael, CA). Data are presented as means ± SE. For the acute study, values before and after administration of the test substance were compared using Student’s paired t-test. For the acute study where more than two groups were examined, comparisons between the groups were analyzed using one-way ANOVA. For the chronic study, differences between control and HGF-treated rats were analyzed by Student’s t-test. A value of P < 0.05 was considered significant.

RESULTS

Acute HGF-induced decline in GFR is prevented by endothelin receptor blockade. To determine the acute actions of HGF on renal hemodynamics, we studied six groups of rats (Table 1), three control groups and three groups of rats that were given a bolus injection of HGF. There were no significant differences in mean baseline values for body weight, MAP, hematocrit, GFR, RPF, and filtration fraction among the six groups (Table 2). To determine whether the dose of bosentan that we employed was sufficient to block the hemodynamic effects of ET-1, we examined the effects of exogenous ET-1 in rats (BOS-ET) that were pretreated with bosentan by gavage 1 h before the start of the study. There were no significant changes in MAP (120 ± 1 h before the start of the study. To determine whether the dose of bosentan that we employed was sufficient to block the hemodynamic effects of ET-1, we examined the effects of exogenous ET-1 in rats (BOS-ET) that were pretreated with bosentan by gavage 1 h before the start of the study. There were no significant changes in MAP (120 ± 2 pre, 121 ± 4 mmHg post), GFR (0.088 ± 0.08 pre, 0.80 ± 0.11 ml/min post), or RPF (4.03 ± 1.31 pre, 3.73 ± 1.36 ml/min post) after infusion of ET-1, demonstrating that bosentan as administered completely blocked the vasoconstrictor effects of ET-1 in our model. CON and BOS-CON rats received only a saline bolus between periods 1 and 2 and served as time controls. As expected, there were no significant changes in any parameter over the course of the study in these two groups (data not shown). Hematocrit also did not change significantly during the course of the study in any group.

Plasma and urine concentrations of HGF were measured using ELISA at different time points after vehicle or HGF injection. As shown in Fig. 1, plasma HGF levels increased markedly from a basal level of 0.38 by ~100-fold, peaking at ~30 ng/ml 15 min after the injection, and remained elevated for 120 min. As expected, plasma HGF concentration in controls was low (0.5 ng/ml) and remained constant throughout the study. To demonstrate that HGF in the plasma was delivered to the kidney, we measured urine HGF levels over the course of the study in several rats. As shown in Fig. 2, urine HGF concentration also increased significantly, from a basal level of 0.39, peaking at 2.5 ng/ml 30 min after injection, and remaining elevated until 240 min after injection, the last time point measured. Urine HGF concentration in control rats was always <0.3 ng/ml.

Acute administration of HGF was associated with significant changes in systemic and renal hemodynamics. The effects of HGF on MAP in HGF, BOS-HGF, and CAN-HGF rats are summarized in Fig. 3. In rats that received no pretreatment, blood pressure tended to decline, from a mean value of 115 ± 4 to 111 ± 4 mmHg after HGF; however, this change did not quite reach statistical significance (P = 0.08). However, an enhanced vasodilator response was observed after pretreatment with bosentan. MAP fell from 110 ± 5 to 103 ± 4 mmHg (P < 0.003) in BOS-HGF rats. In contrast, baseline blood pressure...
tended to be lower in rats pretreated with candesartan (Table 2, \( P = 0.08 \)) but did not decline in response to HGF. The effects of HGF on GFR are summarized in Fig. 4. Administration of HGF was associated with a prompt 30% reduction in GFR in normal rats, from 1.12 ± 0.08 to 0.78 ± 0.08 ml/min (\( P < 0.002 \)). Of note, the HGF-induced decline in GFR was completely prevented by bosentan. Rats pretreated with candesartan displayed a significant (\( P = 0.02 \)) but somewhat blunted 14% decline in GFR in response to HGF. There were no significant changes in RPF in any group, suggesting that the reduction in GFR did not result from alterations in glomerular capillary plasma flow rate (not shown). A reduction in GFR at constant plasma flow must result in a decrease in the calculated value for the filtration fraction, which declined significantly in HGF rats from 0.28 ± 0.03 to 0.20 ± 0.03 (\( P < 0.03 \)) but was not significantly altered in the other two HGF-treated groups.

**HGF induces ET-1 production in glomerular endothelial cells.** Our in vivo studies suggested that HGF induced the production of ET-1 acutely and that altering ET-1 production affected renal hemodynamics. Prior data demonstrates that HGF induces a decline in ET-1 levels at 48 h; therefore, we examined the acute effects of HGF treatment on ET-1 production. We used bovine glomerular endothelial cells to test our hypothesis, that acute treatment with HGF induces release of ET-1. Figure 5A demonstrates that there is a fourfold induction of ET-1 production at 30 min after treatment with HGF. ET-1 production in response to HGF plateaus at 1–2 h, followed by a decline in ET-1 levels below baseline at 4 and 8 h (both of which are statistically significant). Furthermore, a dose-response of ET-1 production in response to HGF depicted in Fig. 5B illustrates that, while there is some increase in ET-1 production after treatment with 1 ng/ml HGF, there is a four- to fivefold increase on treatment with 10–100 ng/ml HGF. There is a correlation between treatment with 10 ng/ml and...
plasma levels of HGF that were noted in rats chronically treated with HGF, suggesting that our findings in cultured endothelial cells might closely represent the in vivo situation in rats treated with bolus doses or chronic infusion of HGF. Moreover, these data imply that HGF regulates ET-1 release and chronic production.

**Chronic treatment with HGF suppresses ET-1 levels and GFR in vivo.** To determine whether the HGF-induced reduction in GFR was sustained when circulating HGF levels were tonically elevated, studies were performed in rats that received a continuous infusion of HGF or vehicle intravenously for 1 wk. As shown in Fig. 6, the HGF infusion produced a sustained 5- to 10-fold increase in plasma HGF concentration assessed by ELISA on days 2, 5, and 7 of the infusion. As shown in Table 3, there were no significant differences in body weight or hematocrit between the two groups. Once again, MAP tended to be lower in the HGF-treated group; however, this difference was not statistically significant ($P = 0.23$) due to much variability in blood pressure within each group. Of note and similar to the acute study, chronic administration of HGF was associated with a significant (50%) decline in GFR. Although HGF stimulates proliferation of renal tubular cells in vivo, there was no difference in kidney weight between the two groups. Renal morphology as assessed by light microscopy was also completely normal in the HGF-treated rats (data not shown).

Immunohistochemical analysis was used to examine the effects of the sustained infusion of HGF on renal ET-1 levels. As shown in Fig. 7, B, C, E, and F, ET-1 was detected in the glomerular and tubulointerstitial compartments of both HGF-infused and control rats. Figure 7, A and D, shows negative controls. However, after 1 wk of HGF infusion, there was a noticeable reduction in staining for ET-1 in HGF-treated rats (Fig. 7, C and F, compared with B and E). To quantify the intensity of ET-1 staining in kidneys of rats treated with HGF for 1 wk, a blinded observer examined and scored ET-1 staining in the glomeruli and interstitium as detailed in METHODS. Figure 8 demonstrates that treatment of rats with HGF for 1 wk resulted in statistically significant decreased staining for ET-1 in both the glomeruli and interstitium. This confirms our in vitro findings of decreased ET-1 production and supports our notion that some of the beneficial effects of HGF on the kidney might be by a chronic decrease in ET-1 levels.

**DISCUSSION**

HGF is a multifunctional cytokine that regulates diverse cellular processes, including proliferation, differentiation, motility, and survival. In the kidney, the HGF/met axis has most often been viewed as a paracrine pathway in which HGF is produced by mesenchymal cells such as fibroblasts and then exerts its actions by binding to its receptor met on renal tubular epithelial cells. However, in addition to tubular cells, c-met is also abundantly expressed on endothelial cells. Studies of the effects of HGF on endothelial cell function have largely focused on its role as an angiogenic factor (37). Similar to findings in renal epithelial cells, HGF has been shown to promote endothelial cell proliferation, migration, protease production, and differentiation into capillary-like structures. However, whether HGF also has significant hemodynamic effects has hardly been examined, and its effects on renal hemodynamics are essentially unknown.

In one study, Yang et al. (45) administered acute intravenous injections of 10 to 625 μg/kg of recombinant HGF to normal rats and monitored systemic hemodynamics. They observed an immediate, significant reduction in MAP of ~8 mmHg with doses above 100 μg/kg, which was associated with a drop in cardiac output and an increase in heart rate. MAP declined by ~4 mmHg in rats that received the low 10 μg/kg dose; however, this was not statistically significant. The hemodynamic changes were transient and resolved within 30 min. The HGF response was blocked when rats were pretreated with a NO synthase inhibitor that induced a large initial increase in blood pressure.

We also observed a small decline in blood pressure in response to HGF, and this decline was accentuated in the presence of the ET-1 receptor antagonist bosentan. These data suggest that the effects of HGF on vascular responses are complex, involving the release of not only relaxing factor(s) such as NO but also the potent vasoconstrictor ET-1. That HGF induced ET-1 release was confirmed in vitro by incubating glomerular endothelial cells with HGF. In fact, a biphasic response was noted. After addition of HGF, ET-1 release was enhanced initially, after 30 min of exposure, consistent with the rapid changes in blood pressure and GFR that we observed. Levels of ET-1 produced in response to HGF remained elevated for up to 2 h, followed by a decrease in ET-1 to less than control levels. Moreover, ET-1 production from BGE increased in a dose-dependent fashion in response to HGF, suggesting that the met receptor phosphorylation is likely involved in this process. Immunostaining of kidneys of rats that received HGF for 1 wk confirmed that the net effect of

**Table 3. Effects of an intravenous infusion of HGF for 1 wk**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>MAP, mmHg</th>
<th>Hct, %</th>
<th>GFR, ml/min × 100 g⁻¹</th>
<th>Kidney Wt/Body Wt, g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>223±12</td>
<td>113±4</td>
<td>40±1</td>
<td>0.87±0.17</td>
<td>0.46±0.03</td>
</tr>
<tr>
<td>HGF</td>
<td>197±12</td>
<td>106±4</td>
<td>39±1</td>
<td>0.43±0.04</td>
<td>0.46±0.03</td>
</tr>
<tr>
<td>$P$ value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>$&lt;0.05$</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. NS, not significant.
prolonged exposure was mild suppression of ET-1 production. It should be noted that suppression of ET-1 with long-term exposure to HGF is in keeping with observations made previously in rabbit proximal tubule cells and in various types of endothelial cells (14–17).

The most novel and striking finding in the present study is that intravenous administration of HGF was associated with an immediate 30% reduction in GFR in normal rats and that this effect was sustained up to 1 wk when HGF was infused continuously. Acutely, the effect of HGF to reduce GFR could be completely prevented by pretreatment with the mixed ETA/ETB endothelin receptor antagonist bosentan, demonstrating that it is largely, if not entirely, mediated by HGF-induced release of this potent vasoconstrictor. However, because ET-1 levels declined to values below normal by 2 h after exposure to HGF and remained modestly suppressed at 7 days in renal tissue of HGF rats, another mechanism must account for the reduction in GFR observed in rats that received the sustained infusion. Alternatively, it is attractive to speculate that the initial release of ET-1 in response to HGF induces an upregulation of endothelin receptors, rendering the vasculature exquisitely sensitive to the low levels of local ET-1 and thus sustaining decreased GFR. Additional studies examining the effects of blocking various vasoactive substances in the chronic setting are needed to resolve this issue.

ET-1 is a crucial regulator of vascular tone and an important mediator of a variety of biological functions. It is produced primarily by endothelial cells; however, other cell types such as smooth muscle cells and proximal tubular epithelial cells (34, 46) also produce ET-1. ET-1, presumably released from damaged endothelium, may play an important intermediary role in the hypoperfusion and hypofiltration observed in postischemic kidneys (20). Chronic treatment of proximal tubular epithelial cells and endothelial cells with HGF results in a
The HGF-induced reduction in GFR occurred in the absence of any decline in RPF rate, suggesting that it resulted either from a decrease in glomerular capillary pressure (P_GC) or in the ultrafiltration coefficient (Kf). Due to autoregulation, the modest decline in systemic blood pressure we observed is extremely unlikely to have led to a significant reduction in P_GC. A reduction in P_GC has been observed in one study after administration of exogenous ET-1 to rats; however, this was associated with an increase in afferent and efferent arteriolar resistance and a decline in plasma flow rate (20). Therefore, a reduction in P_GC is unlikely to be responsible for the decline in GFR observed here. ET-1-induced reductions in RPF rate, suggesting that, at least in part, the effects of HGF on vascular and renal tissue may be mediated by alterations in ET-1 production and in ET-1 action as well. Thus ANG II has been reported to induce ET-1 expression in endothelial and vascular smooth muscles cells, and in vitro. It is reported that ANG II can suppress endogenous ET-1 production, such as we observed previously demonstrated that HGF can reduce injury, ameliorate progression of interstitial fibrosis in rats with established renal injury. Kidney Int 64: 409–419, 2004.


HGF REDUCES GLOMERULAR FILTRATION RATE

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