IGF-I inhibitors reduce compensatory hyperfiltration in the isolated rat kidney following unilateral nephrectomy

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Abstract
Background. A role for insulin-like growth factor-I (IGF-I) as a mediator of renal hyperfiltration and hyperperfusion following unilateral nephrectomy (UNx) has been examined.

Methods. Adult male Wistar rats were subjected to either left UNx or sham operation. Twenty one days after surgery, the right kidney was removed under barbiturate anaesthesia, and renal function was measured ex vivo using an isolated rat kidney perfusion system. The glomerular filtration rate was assessed from the renal clearance of $^{14}$C-inulin.

Results. UNx stimulated renal growth as shown by a significantly higher ($P<0.02$) tissue dry weight in kidneys from UNx (0.45 ± 0.02 g) than from sham-operated rats (0.31 ± 0.02 g). Compensatory hyperfiltration could be detected ex vivo; kidneys obtained from UNx rats having a significantly higher ($P<0.05$) $[^{14}]$C-inulin clearance (0.75 ± 0.08 ml/min, $n=8$) than kidneys obtained from sham-operated animals (0.39 ± 0.05 ml/min, $n=8$). Compensatory hyperperfusion was also detected ex vivo; kidneys obtained from UNx rats having a significantly higher ($P<0.05$) renal perfusate flow (28.2 ± 2.7 ml/min) than kidneys obtained from sham-operated rats (22.5 ± 0.8 ml/min). Following perfusion with either 50 μg monoclonal IGF-I antibody ($n=4$) or 6.5 μM genistein ($n=4$), an inhibitor of tyrosine kinase, no significant difference in $^{14}$C-inulin was observed between kidneys obtained from either UNx or sham-operated rats. In contrast to hyperfiltration, renal hyperperfusion remained unaffected by the IGF-I antibody and was only reduced by 30% following genistein administration.

Conclusions. The results suggest a role for renal IGF-I as a mediator of compensatory hyperfiltration in the rat.

Key words: IGF-I; hyperfiltration; uninephrectomy; isolated kidney; genistein; glomerular filtration rate

Introduction

Growth hormone (GH) and insulin-like growth factor I (IGF-I) have both been proposed as mediators of the renal growth which follows a reduction in renal mass [1,2]. GH and IGF-I also stimulate kidney function increasing renal blood flow and the glomerular filtration rate (GFR) [3,4], indicating a second possible role as mediators of renal hyperfiltration and/or hyperperfusion. Micropuncture studies show that IGF-I, although a renal vasodilator, elevates the GFR through an increase in the ultrafiltration coefficient ($K_f$) [5] rather than an increase in glomerular hydrostatic pressure. Since glomerular hyperfiltration following unilateral nephrectomy (UNx) can easily be demonstrated in the Lewis dwarf rat, a strain deficient in GH [6], experiments have been performed to examine the involvement of IGF-I as a mediator of compensatory hyperfiltration and/or hyperperfusion.

Renal function was measured ex vivo using an isolated perfused rat kidney preparation (IPRK). The vasodilated state of the IPRK ensured the presence of filtration disequilibrium allowing changes in GFR produced by alterations in $K_f$ to be detected [7]. An increase in GFR in response to exogenous recombinant human IGF-I has already been demonstrated in this IPRK model [8]. Renal function was measured in kidneys obtained from rats subjected to either UNx or sham operation, 21 days after surgery. The influence of two IGF-I inhibitors was examined, a neutralizing IGF-I antibody [9] and genistein, an inhibitor of tyrosine kinase [10]. Tyrosine kinase is involved in the early stages of IGF-I signal transduction [11].

Materials and Methods

Male Wistar rats (330–350 g) were housed 2–3 per cage at an ambient temperature of 20–22°C, humidity of 45% and light/dark cycles of 12 h, and had free access to standard animal chow (protein/casein content 18%) and water. UNx was undertaken under ether anaesthesia. The left kidney was exposed through a flank incision, the adrenal gland was separated from the upper pole and the kidney was decapsulated. The renal pedicle was ligated and the kidney removed.
Sham-operated rats underwent a similar flank incision. The right kidney was perfused ex vivo and renal function measured 21 days after surgery. All procedures were undertaken according to the regulations laid down by HM Government (Animals Scientific Procedures Act, UK 1986).

**Isolated perfused rat kidney (IPRK)**

Rats were anaesthetized with 125 mg/kg thiopentone, and the kidney was removed by a non-ischaemic technique [8]. Following an intravenous injection of heparin (1000 U/kg), the right ureter was cannulated and the right adrenal artery ligated. The right renal artery was cannulated via the superior mesenteric artery, perfusion was started in situ and the right kidney was transferred to a recirculating perfusion system. The perfusate consisted of a modified Krebs-Henseleit solution containing 3.5% polygeline gassed with 95% O₂/5% CO₂ and maintained at 37°C. Perfusion pressure was measured from a point inside the renal artery and was maintained at 100 mmHg by a servo-controlled pump. Renal perfusate flow (RPF) was monitored continuously from the speed of the servo-pump.

**Experimental protocol**

Kidneys were perfused for a period of 90–120 min. The first 50 min was regarded as an equilibration period during which time a bolus dose of [¹⁴C]inulin (1 µCi) was added to the perfusion fluid for the serial measurement of [¹⁴C]inulin clearance. Perfusate and urine samples were collected at 5 min intervals for the serial measurement of [¹⁴C]inulin clearance, baseline values being obtained in a 50–60 min period of perfusion. Inhibitors were added to the perfusion system at 60 min. Genistein was added as a bolus dose giving an initial perfusate concentration of 6.5 µM [10], samples being collected for a 30 min period after addition of the drug to the perfusate. IGF-I antibody (anti-human, monclonal IgG₅₁) or mouse IgG₅₁ were dissolved in phosphate-buffered saline and 50 µg [9] was infused into the perfusion circuit over a 10 min period using a syringe pump (50 µg/ml, 100 µl/min). Delivery syringes and tubing were primed with 1% bovine serum albumin to avoid antibody adherence. For studies with the IGF-I antibody, samples were collected for a 60 min period following its addition to the perfusate.

**Antibody neutralization**

Two days prior to assay, opossum kidney (OK) cells were seeded in Dulbecco’s modified Eagle’s medium (DMEM) containing 1-glutamine, 5% fetal calf serum (FCS), 100 IU/ml penicillin and 100 µg/ml streptomycin. All cultures were maintained at 37°C in a humidified atmosphere of 5% CO₂ in air. Cells were plated in 75 cm² tissue culture flasks. One day prior to assay, cells were serum starved. On the day of the assay, the culture medium was replaced by medium which had been pre-incubated for 1 h with either recombinant human IGF-I (15 ng/ml) alone or together with an increasing concentration of IGF-I antibody (0–80 µg/ml). Cells were incubated for 24 h.

Following this incubation, the culture medium was removed and cells were pulsed with medium containing 2 µCi/ml [³H]thymidine and 0.5% FCS for 4 h. Cells were washed three times with 4 mM thymidine and incubated with 10 ml of ice-cold, 5% trichloroacetic acid (TCA) for 90 min followed by the addition of 4 ml of 0.5 M NaOH for a further 2 h period. Samples from the supernatant were counted for [³H].

**Materials**

Genistein was from Calibiochem, mouse anti-human IGF-I antibody from Upstate Biotechnology, TCS Biochemicals, UK, and recombinant human IGF-I from Kabo, Sweden. All other materials including mouse IgG₁ were obtained from Sigma.

**Statistical analysis**

Results were expressed as mean ± SEM. Students t-test and linear regression analysis were performed using Excel 5.1 software.

**Results**

**Hyperfiltration and hyperperfusion in the IPRK**

The [¹⁴C]inulin clearance and renal perfusate flow determined in kidneys obtained from UNx rats were measured in the presence of established renal growth. Kidneys obtained from UNx rats had a significantly higher dry weight (0.31 ± 0.02 g vs 0.45 ± 0.02 g, n = 8 per group, P < 0.02) and higher content of total protein (128 ± 12 mg vs 160 ± 9 mg, n = 8 per group, P < 0.01) than kidneys obtained from sham-operated animals, representing an increase in renal growth of some 33%. The renal hyperfiltration and hyperperfusion measured following UNx is shown in Figure 1. [¹⁴C]Inulin clearance was significantly higher (P < 0.01) in kidneys obtained from UNx rats (0.75 ± 0.08 ml/min, n = 8) than from sham-operated animals (0.39 ± 0.05 ml/min, n = 8). This represented an increase of 92%. The renal perfusate flow was also significantly higher (P < 0.05) in kidneys obtained from UNx rats (28.2 ± 2.7 ml/min, n = 8) than from sham-operated animals (22.5 ± 0.8 ml/min, n = 8). This represented an increase of 29%, a similar change to the increase in kidney growth.

![Fig. 1. [¹⁴C]Inulin clearance, renal perfusate flow and the dry weight of kidneys perfused ex vivo at constant pressure (100 mmHg) obtained from either sham-operated (unhatched) or UNx (hatched) rats, 21 days after surgery. n = 8 per group.](image-url)
Antibody neutralization

The ability of the IGF-I antibody to neutralize a biological response to exogenous recombinant IGF-I (rhIGF-I) in a kidney cell line is shown in Figure 2. RhIGF-I (15 ng/ml) produced a 100% increase in the incorporation of radiolabelled thymidine into DNA, an effect entirely prevented by the IGF-I antibody at 60 μg/ml.

IGF-I inhibitors and renal hyperfiltration

The effect of the IGF-I antibody (50 μg) on [14C]inulin clearance in the IPRK is shown in Table 1. Prior to antibody administration, [14C]inulin clearance was significantly higher in kidneys obtained from UNx rats than sham-operated animals (P<0.05). However, by the 50–60 min period following antibody administration, no significant difference in [14C]inulin clearance between kidneys obtained from UNx rats and sham-operated animals was apparent. Renal hyperfiltration is shown in Figure 3 as the difference in mean [14C]inulin clearance between kidneys obtained from UNx and sham-operated animals (n=4 per group). One hour after the addition of IGF-I antibody to the renal perfusate, renal hyperfiltration had decreased with time (R=0.8939, P<0.01) to 47% of its original value without reaching a new stable baseline. Perfusion of kidneys obtained from UNx rats with 50 μg of mouse IgG1, produced no change in [14C]inulin clearance (0.82±0.14 vs 0.85±0.09 ml/min, n=4). [14C]Inulin clearance in kidneys obtained from sham-operated animals was entirely unaffected by IGF-I antibody administration.

The effect of 6.5 μM genistein on [14C]inulin clearance is also shown in Table 1. Prior to genistein administration, [14C]inulin clearance was significantly higher in kidneys obtained from UNx rats than sham-operated animals (P<0.05). However, by the 20–30 min period following genistein administration, no significant difference in [14C]inulin clearance between kidneys obtained from UNx rats and sham-operated animals was observed. In the 30 min period after the addition of genistein to the renal perfusate, [14C]inulin clearance decreased with time (R=0.9974, P<0.005), renal hyperfiltration being abolished.

![Fig. 2. Neutralization curve for mouse monoclonal IGF-I antibody against the increase in [3H]thymidine incorporation induced by recombinant human IGF-I (15 ng/ml) in opossum kidney cells. Each data point represents a mean of two experiments.](image)

![Fig. 3. Effect of a neutralizing IGF-I antibody (mouse, anti-human 50 μg) on renal hyperfiltration and hyperperfusion following 21 day unilateral nephrectomy. Renal hyperfiltration (●) or renal hyperperfusion (○) were calculated as the difference between the mean [14C]inulin clearance or the mean renal perfusate flow, respectively, measured in groups of kidneys (n=4 per group) obtained from either UNx or sham-operated rats.](image)

Table 1. Effect of a neutralizing IGF-I antibody (50 μg) and a tyrosine kinase inhibitor, genistein (6.5 μM) on [14C]inulin clearance and renal perfusate flow in kidneys obtained from UNx and sham-operated rats, 21 days after surgery

<table>
<thead>
<tr>
<th>IGF-I antibody</th>
<th>Control</th>
<th>Experimental</th>
<th>Renal perfusate flow</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>(50 μg)</td>
<td>Sham</td>
<td>0.37±0.04</td>
<td>0.31±0.10</td>
<td>21.8±1.0</td>
<td>24.0±0.7</td>
</tr>
<tr>
<td></td>
<td>UNx</td>
<td>0.78±0.14*</td>
<td>0.38±0.07</td>
<td>26.7±2.6*</td>
<td>28.3±2.3*</td>
</tr>
<tr>
<td>Genistein</td>
<td>Sham</td>
<td>0.41±0.07</td>
<td>0.38±0.07</td>
<td>23.1±0.5</td>
<td>26.9±0.3</td>
</tr>
<tr>
<td>(6.5 μM)</td>
<td>UNx</td>
<td>0.73±0.03*</td>
<td>0.37±0.07*</td>
<td>29.7±2.9*</td>
<td>31.7±3.3*</td>
</tr>
</tbody>
</table>

Experimental measurements were taken 55 mins after the addition of the antibody and 25 mins after the addition of genistein to the renal perfusate (n=4 per group). *P<0.05.
(Figure 4). Genistein administration did not produce a significant fall in $[^{14}C] $inulin clearance in kidneys obtained from sham-operated animals.

**IGF-I inhibitors and renal hyperperfusion**

The effect of IGF-I antibody on renal perfusate flow is shown in Table 1. Prior to antibody administration, renal perfusate flow was significantly higher in kidneys obtained from UNx rats than sham-operated animals ($P<0.05$). This difference remained throughout the period of kidney perfusion, the renal perfusate flow increasing with time in kidneys obtained from both sham-operated and UNx rats. Renal hyperperfusion is shown in Figure 3 as the difference in mean renal perfusate flow between kidneys obtained from UNx and sham-operated animals ($n=4$ per group). Despite the reduction in hyperfiltration, renal hyperperfusion remained unaffected by IGF-I antibody administration. Perfusion of kidneys obtained from UNx rats with 50 μg of mouse IgG$_{1}$ produced no change in renal perfusate flow ($25.9 \pm 3.2$ to $28.1 \pm 3.5$ ml/min, $n=4$).

The effect of genistein on renal perfusate flow is also shown in Table 1. Prior to genistein administration, renal perfusate flow was significantly higher in kidneys obtained from UNx rats than sham-operated animals ($P<0.05$). This difference remained throughout the period of perfusion, the renal perfusate flow increasing with time in kidneys obtained from both sham-operated and UNx rats. The effect of genistein on renal hyperperfusion is shown in Figure 4. In contrast to the abolition of hyperfiltration, hyperperfusion showed a modest but significant ($R=0.8938$, $P<0.01$) decline with time of $\sim 30\%$.

**Discussion**

The detection of renal hyperfiltration and hyperperfusion following UNx in kidneys perfused ex vivo suggested the involvement of intrarenal mechanisms rather than a systemic response. The extent of hyperfiltration ($\sim 90\%$) detected 21 days following UNx was similar to that reported from in vivo studies in the Munich–Wistar [12] or the Lewis rat [6] where renal function was measured under barbiturate anaesthesia. IGF-I mRNA has been isolated from rat glomeruli [13], and the presence of IGF-I protein in the glomerulus has been detected recently by immunohistochemistry [14], the mesangial cell being the major site of production [15]. An increase in vascular load, such as might be expected following UNx, also increases the expression of IGF-I in blood vessels [16]. The use of the IPRK to study mechanisms associated with renal hyperfiltration avoided both the measurement of apparent hyperfiltration due to an increased blood volume [17] and changes in renal function being generated indirectly from the systemic effects of drug inhibitors or antibodies. Changes in IGF-I biochemistry following UNx previously have been associated with the development of compensatory growth rather than hyperfiltration [1,2]. However, the involvement of IGF-I in the present study could not be attributed to kidney growth since IGF-I inhibitors were administered to the kidney within the perfusion system, ex vivo.

In the present IPRK study, renal hyperfiltration was reduced by a neutralizing IGF-I antibody and abolished by genistein, suggesting a mediator role for IGF-I. As expected, the effect of the IGF-I antibody was relatively slow, with hyperfiltration still declining after a 60 min perfusion period in the absence of changes in renal hyperperfusion. Hyperfiltration was abolished after a 30 min perfusion period with genistein, a non-selective inhibitor of tyrosine kinase [10]. Tyrosine kinase is an enzyme involved in the early stages of signal transduction following activation of the IGF type I receptor [11]. Neither the neutralizing antibody nor genistein reduced the GFR or RPF in kidneys obtained from sham-operated animals. The modest inhibition of hyperfiltration induced by genistein but not by the IGF-I antibody may be explained either by the involvement of tyrosine kinase in the control of vascular smooth muscle contractility [18] or by other pharmacological properties of genistein. The mechanism of renal hyperperfusion would therefore appear to be independent of hyperfiltration, and within our IPRK system has yet to be separated from kidney growth.

The IGF-I antibody employed was a monoclonal anti-human IGF-I (class IgG$_{1}$) raised in the mouse [9]. Control studies using a mouse immunoglobulin (IgG$_{1}$) showed no effect on GFR in perfused kidneys from UNx rats. The ability of the antibody to neutralize the biological response to IGF-I was examined in OK cells where the increase in thymidine incorporation induced by recombinant human IGF-I was completely neutralized, consistent with previous studies in rat osteoblasts [19]. In the IPRK, at 50 μg, the IGF-I antibody dose was considered to be in excess of that required to neutralize the effect endogenous IGF-I generated in the renal glomerulus. In a perfusion
system it is the total amount of peptide available for neutralization which is the important criterion in determining antibody dose. The rat kidney contains some 200 ng of IGF-I [20], most of which is bound in the distal nephron, unavailable for neutralization by the antibody employed.

IGF-I is well known to increase GFR in both man [4] and the rat [21] while recombinant human IGF-I previously has been demonstrated to increase GFR in a similar IPRK model to that used in the present study [8]. Mechanisms associated with the renal effects of IGF-I include stimulation of renal autacoids such as nitric oxide (NO) [22], bradykinin [23] or prostaglandins [21]. The ability of IGF-I to stimulate NO synthesis directly has been demonstrated previously in endothelial cell culture, where the increase in NO induced by IGF-I was blocked by the tyrosine kinase inhibitor genistein [24]. In previous IPRK studies, we have demonstrated that the hyperfiltration induced by UNx can be prevented by the NO synthase inhibitor t-NAME [25]. The IGF-I analogue, des-1,3 IGF-I, elevated single nephron GFR (SNGFR) in the dwarf rat through an increase in the ultrafiltration coefficient \( K_f \) in the presence of a decrease in glomerular hydrostatic pressure [5]. Due to the inherent vasodilated condition of the IPRK, the glomerular capillary is likely to be in filtration disequilibrium, a state where the GFR can be altered by changes in \( K_f \) [7]. NO could enhance the \( K_f \) by increasing the surface area of either the glomerular capillary endothelium through mesangial cell relaxation or the filtration slit diaphragm through relaxation of cytoskeletal proteins in the podocyte [26]. Micropuncture studies in the Munich–Wistar rat originally suggested hyperfiltration following UNx to be mediated through an increase in glomerular hydrostatic pressure [12]. More recent experiments using young nephrectomized rats demonstrated that a high ultrafiltration pressure is not an absolute requirement for compensatory hyperfiltration, an increase in \( K_f \) being observed [27]. \( K_f \) is also elevated in the diabetic rat following angiotensin-converting enzyme (ACE) inhibition where glomerular hyperfiltration is sustained, despite normalization of the previously elevated glomerular hydrostatic pressure [28]. In conclusion, studies performed \textit{ex vivo} using the isolated rat kidney support a role for glomerular IGF-I as a mediator of the hyperfiltration which follows unilateral nephrectomy.  

Acknowledgements. We are indebted to Baxter Health Care and the National Kidney Research Foundation (Grant R/41/1/98) for their financial support.

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Received for publication: 22.12.99
Accepted in revised form: 26.8.99