

# Studies on the Renal Kinetics of Growth Hormone (GH) and on the GH Receptor and Related Effects in Animals

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## ABSTRACT

Growth hormone (GH) is filtered through the kidney, and may exert effects on renal function when presented via the circulation. Investigations on kidney-related aspects of GH are increasing in number. Using *in vitro* and *in vivo* approaches, the present study attempted to provide answers to a number of unresolved or debated issues. *In vitro*, we detected both GH and type 1 IGF receptors (R) in a porcine renal epithelial cell line. The saturation and down regulation kinetics of the GH-R indicate that it has the properties of a classical GH-R. Furthermore, the simultaneous presence of GH-R and IGF-R on a phenotypically homogeneous cell line suggests the presence of GH-induced auto-/paracrine IGF-I bioactivity in the kidney. Experiments with isolated proximal rabbit tubules incubated with physiological concentrations of <sup>125</sup>I-GH demonstrated a time- and dose-dependent increase in unlabelled GH-displaceable cell-associated radioactivity, lending support to the concept of GH mediating its renal effects via proximal tubular GH-R. Short term administration of GH to rats and humans elicited electrolyte and water retention that may cause edema in adults. In the present study, long term administration of GH to rats caused only a minor increase in serum phosphate levels, with no changes observed in the renal electrolyte clearance. During the first 4 days of GH treatment in rats, no change in plasma renin activity was detected and we were thus unable to confirm the hypothesis that the renin-angiotensin system is responsible for the early phase of GH-associated fluid retention. Pharmacokinetically, when GH was admin-

istered to rats with functional disconnection of the kidneys as a model of renal insufficiency, the whole body clearance of GH decreased by ca. two thirds, and was reflected by an increase in the mean residence time and AUC<sub>plasma</sub> for GH. The plasma half-life, however, was not significantly affected, suggesting that the volume of distribution (Vd) had decreased for the GH administered to the renally compromised animals. A renal contribution to the Vd was visualized as intense radioactive staining in the kidney region on whole body autoradiographs (WBA) of rats dosed with <sup>125</sup>I-labelled hGH. The liver region was also intensely stained. Kidney-associated radioactivity was found to be related not only to glomerular filtration, but also to peritubular uptake, since the renal clearance of free GH was found to exceed the GFR. The overall conclusion from this study is that the kidneys contribute significantly to GH elimination, and that renal GH elimination probably involves uptake and degradation mediated by GH-R that appears similar to the classical human receptor.

## KEY WORDS

growth hormone, receptors, IGF-1, pharmacokinetics, renal clearance, fluid retention, plasma renin activity

## INTRODUCTION

It has been known for almost half a century that the kidney is not only involved in the removal of growth hormone (GH) from the circulation, but also

acts as a target organ for this hormone /1/. In fact, GH exerts a number of effects on renal function and the mechanisms involved have recently been partially delineated /2/. Focusing on the known pharmacodynamic and pharmacokinetic associations between GH and the kidneys, the present paper attempts to provide answers to some unresolved issues. These issues appear relevant since, clinically, GH is receiving increasing attention as an anabolic agent in children with chronic renal insufficiency (CRI). Accelerated growth has been obtained on administration of GH to such children /3/. In CRI children, secretion of GH is not reduced /4/ but an imbalance between the concentration of free insulin-like growth factor-1 (IGF-1) and IGF-binding proteins probably causes reduced IGF bioactivity /3/, which GH therapy aims at restoring.

Pharmacokinetically, the fraction of GH unbound to the GH-binding protein (GH-BP) in the circulation is available for renal filtration, possibly followed by tubular reabsorption and degradation /5/. Filtered GH encounters the epithelial cells of the nephron from the luminal side, and it is probably resorbed and degraded (amino acid sparing). In contrast, the fraction of GH arriving through the circulation via the vasa recta at the basolateral side of the tubular epithelium appears to stimulate the cells, as evidenced by second messenger production and altered cellular metabolism and transport processes in GH-stimulated cells /2/. Receptors for GH in the basolateral membrane are presumed to be responsible for the observed effects /6/, even though such receptors have not yet been characterized in detail as classical GH receptors. An *in vivo* correlate of the reported localization of the GH receptor (GH-R) at basolateral membrane sites in proximal tubular cells /6/ would be that radiolabelled GH, previously shown to be sequestered in kidney tissue /7/, should accumulate specifically in the renal cortices due to the presence of GH-R in the cortex.

In CRI patients with severely reduced glomerular filtration rate (GFR), urinary excretion of GH is dramatically increased /8/, probably indicating reduced tubular reabsorption of filtered GH. This is not surprising since, in healthy rats, it has been estimated that more than two thirds of a dose of GH is eliminated via the kidneys /9/. There has been controversy as to whether peritubular GH-

R-mediated GH uptake contributes to the overall renal clearance of GH. Combining experiments using renally ligated animals with knowledge of GFR and the binding of GH to GH-BP (and hence lack of access to filtration) will give a clue as to the role of glomerular and peritubular processes in GH elimination.

Apart from affecting glomerular function, it has been demonstrated that GH exerts an antidiuretic and antialluretic effect in rats within hours /10/, but the long term effects of GH on electrolytes have not been studied in detail in the rat. In humans, electrolyte retention /11/ has been suggested by some /12/, but not all authors /13/, to occur following GH interaction with the renin-angiotensin system (RAS), causing an increase in plasma renin activity (PRA) and aldosterone levels. A prerequisite for the involvement of one or more parts of the RAS in GH-induced fluid retention is the existence of a temporal relationship between the effect on the RAS and the elicited electrolyte retention.

In spite of possible species differences, e.g. between man and rat, some of the unanswered questions raised above can only be addressed in animal models. Consequently, the present *in vitro* and *in vivo* experimental study investigated a number of the unresolved pharmacokinetic and pharmacodynamic issues discussed above. The presented data are derived from experiments conducted in intact rats, rabbits and pigs, or using cells from these species.

## MATERIALS AND METHODS

### *In vitro* experiments on the renal GH and IGF receptors

A porcine renal epithelial cell line, LLC-PK1, was used in all experiments at passage numbers between +1 and +20. The LLC-PK1 cells were cultured in RPMI-1640 supplemented with 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamine (2 mM) and sodium bicarbonate (2 g/l), in 5% CO<sub>2</sub> and 95% air at 37°C. The assay buffer used was 100 mM HEPES, 120 mM NaCl, 1.2 mM MgSO<sub>4</sub>, 2.5 mM KCl, 15 mM Na acetate, 10 mM glucose, 1 mM EDTA and

1% BSA, pH 7.4. Medium, sera and antibiotics were obtained from Gibco.

The hGH (22 kDa) and IGF-1 preparation used both for labelling and as a standard was Norditropin (Novo Nordisk A/S, Gentofte, Denmark). The  $^{125}\text{I}$ -hGH and  $^{125}\text{I}$ -IGF-1 preparations had specific activities of 65  $\mu\text{Ci}/\mu\text{g}$  and 1.94  $\mu\text{Ci}/\text{pmol}$ , respectively.

For the GH displacement experiments, cells in late log phase were centrifuged and washed in RPMI-1640 supplemented with 1% fetal calf serum, penicillin (100 U/ml), streptomycin (100  $\mu\text{g}/\text{ml}$ ), L-glutamine (2 mM), sodium bicarbonate (2 g/l) and 15 mM HEPES (spinner medium). The cells were then resuspended in spinner medium and incubated for 3 hours in a spinner culture flask at 30°C and low rotation,  $5 \times 10^5$  cells/ml. After spinner incubation the cells were washed in assay buffer. 200  $\mu\text{l}$  of the cell supernatant was added in 5 ml polypropylene centrifuge tubes to 25  $\mu\text{l}$   $^{125}\text{I}$ -hGH 22 kDa (225 pM) and 25  $\mu\text{l}$  unlabelled hGH (22 kDa,  $3 \times 10^{-7}$  M), to a total volume of 250  $\mu\text{l}$  and a cell density of  $2 \times 10^6$  cells/ml. The tubes were incubated at 30°C for 90 min. Thereafter 200  $\mu\text{l}$  of the cell mixture was layered on top of 50  $\mu\text{l}$  di-N-butyl phthalate and the cells were centrifuged for 1 min in a Microfuge (9000 rpm). The tip of the tube containing the cells was cut off and the radioactivity bound to the cells was measured in a gamma counter (1282 Compugamma, Wallac). The displacement assay for IGF-1 was performed as described for hGH. The concentration used was 0.14 nM  $^{125}\text{I}$ -IGF-1, and for determination of non-specific binding, 0.14  $\mu\text{M}$  unlabelled IGF-1 was added.

For experiments on the saturation of GH-R with hGH, cells were pretreated for 3 hours in spinner culture, washed in assay buffer, and treated as described in the displacement study. Rising concentrations of  $^{125}\text{I}$ -IGF-1 were added with or without unlabelled hGH ( $3 \times 10^{-7}$  M) and the specific binding was measured after 90 min of incubation.

In experiments on the down-regulation of GH-R, the cells were pretreated as described for the association study. LLC-PK1 cells ( $2 \times 10^6$  cells/ml) were preincubated in tubes at 30°C either with or without hGH (450, 230, 45 pM hGH). After 4 h the cells were centrifuged and  $^{125}\text{I}$ -hGH (225 pM) was added with and without high concentrations of

unlabelled hGH ( $3 \times 10^{-7}$  M), and finally incubated for 90 min. The specific radioactivity was measured after centrifugation through di-N-butyl phthalate in a gamma counter.

#### Experiments with isolated rabbit proximal tubules

Renal proximal tubules from healthy rabbits were isolated as previously described /14/. Briefly, the suspensions were obtained by *in situ* perfusion of the kidneys with iron oxide, collagenase and hyaluronidase, followed by purification using a magnet. A concentration of 10  $\mu\text{M}$  unlabelled hGH was chosen in the displacement experiment.

#### Pharmacokinetic experiments in normal and renally ligated rats

The test solution was prepared by dissolving Norditropin 12 I.E. (0.34 I.E./mg) in 3.0 ml distilled water. One ml thereof was further diluted with Norditropin buffer containing 1% BSA to a final concentration of 50  $\mu\text{g}/\text{ml}$ . Twelve Sprague-Dawley male rats weighing approx. 250 g were allocated randomly to two groups. The rats were anesthetized with pentobarbital 50 mg/kg i.p. and a catheter was inserted in the left carotid artery and was kept open with heparin 20 I.E./ml. They were dosed with 50  $\mu\text{g}$  hGH/kg i.v. Two incisions were placed in the right and left flank of the rat, respectively, after which the kidney arteries were ligated in group I and non-ligated in group II (sham-operated). The test solution was then administered in a volume of 1 ml/kg in a tail artery. Blood samples of approx. 250  $\mu\text{l}$  were drawn from the catheter 5 min before dosing and at the following times post-dose: 5, 10, 15, 20, 30, 40, 50, and 60 min. They were collected in heparin stabilized test tubes and centrifuged, after which the plasma was separated by centrifugation and stored at -20°C until analysis. The concentration of hGH was analyzed by ELISA which does not cross-react with rhGH. Arterial blood pressure was measured before and at termination of the study when S-creatinine was also measured.

The plasma concentrations were analyzed pharmacokinetically by use of a non-compartmental model (PCNONLIN). Total body clearance,  $CL_B$ , was calculated by the following equation:  $CL_B =$

Dose / AUC. The results are presented as the mean and S.E. Differences between the two groups were analyzed by the unpaired Student's t-test.

#### Whole body autoradiography

Each rat received a single intravenous bolus injection of 0.1 ml of the formulated  $^{125}\text{I}$ -hGH solution (nominally *ca.* 30  $\mu\text{Ci}$ ) into a tail vein. Two rats were sacrificed by asphyxiation in a rising concentration of  $\text{CO}_2$  at 0.5 hour after dosing. After sacrifice, each animal was rapidly frozen in a bath of isopentane/dry ice and mounted in aqueous 2% carboxymethylcellulose on an Cryopolycut microtome in a cryostat at *ca.* -25°C (Leica). Sagittal sections (15  $\mu\text{m}$ ), at 5 levels between ovaries and the spinal cord, were mounted on adhesive tape and freeze dried before contact with the X-ray film (Kodak X-omat AR International, UK) in light-proof, lead shielded cassettes at *ca.* -20°C /15/. The exposure time was 29 days. The amounts of radioactivity in tissues were assessed by visual inspection.

#### Effects of long-term treatment with GH on electrolytes in rats

Since previous studies in our laboratory have been of short-term treatment, showing a rapid onset of electrolyte retention in rats /10/, we investigated the long-term effect of GH on urine and serum electrolytes (Beckman autoanalyzer) in ovariectomized rats dosed s.c. once daily for half a year with 1 mg/kg hGH.

#### Measurement of plasma renin activity (PRA) in rats

Two daily assessments of PRA, being performed essentially as described previously in detail /16/, preceded the 7-day treatment period with 2 mg/kg hGH, administered s.c. Daily PRA determinations were made prior to hGH administration during the dosing period.

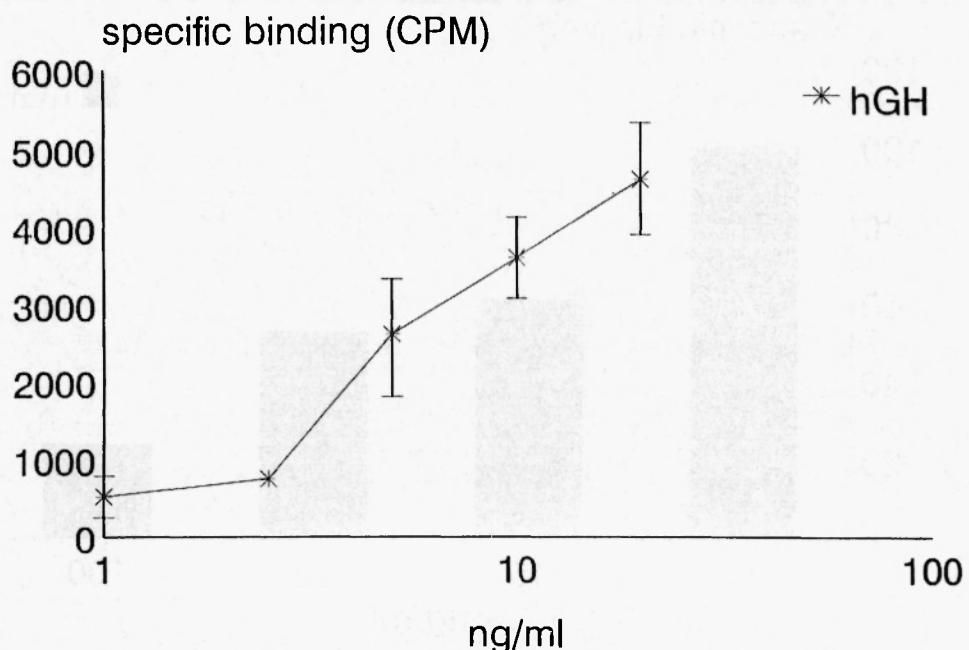
## RESULTS

#### *In vitro* experiments on the renal GH and IGF receptors

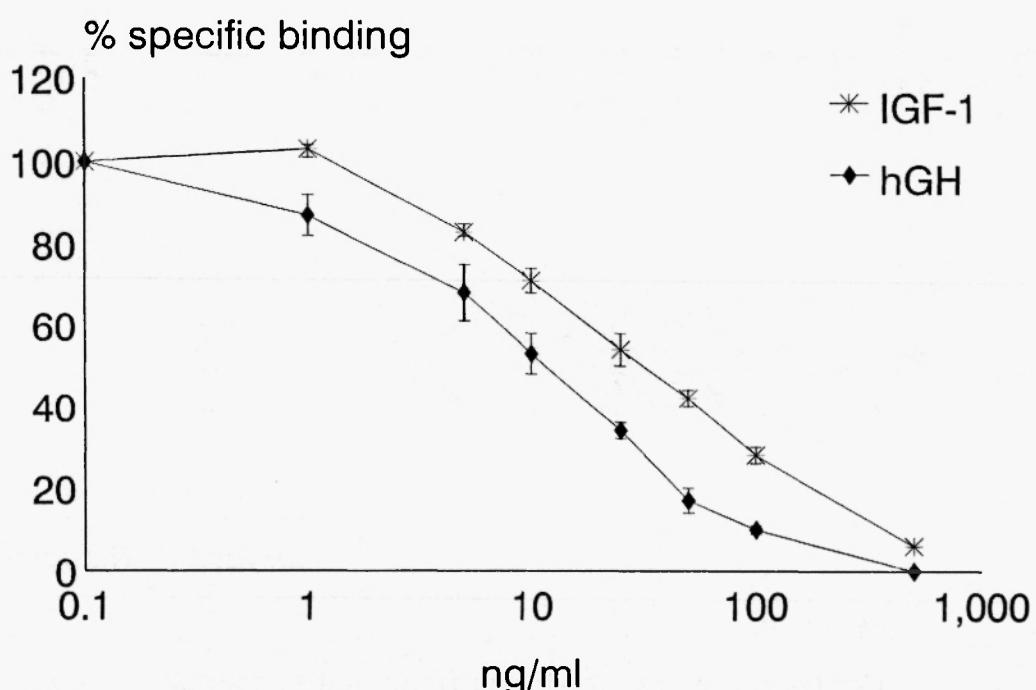
Concentration-dependent high affinity binding of GH - well within the physiological range - occurred when  $^{125}\text{I}$ -hGH was added to the porcine renal epithelial cell line, LLC-PK1 (Fig. 1). Furthermore, displacement of  $^{125}\text{I}$ -hGH with increasing concentrations of unlabelled hGH yielded an  $\text{IC}_{50}$  (concentration of unlabelled hGH causing half-maximal displacement) of approx. 0.5 nM (Fig. 2). the simultaneous presence of GH and IGF receptors on the cells was also suggested by the finding of an  $^{125}\text{I}$ -IGF-1 displacement curve well within the physiological range (Fig. 2). Finally, when cells were preincubated for 4 h with hGH, concentration-dependent down-regulation of the receptor occurred, indicating the presence of a dynamic GH receptor population (Fig. 3). The results imply that classical GH receptors are present on the cells, and these may coexist with receptors for IGF-1, suggesting the possibility that an auto-/paracrine GH/IGF-1 pathway is active within a population of kidney cells such as those described here.

#### Kinetic studies on GH in isolated proximal tubules from rabbits

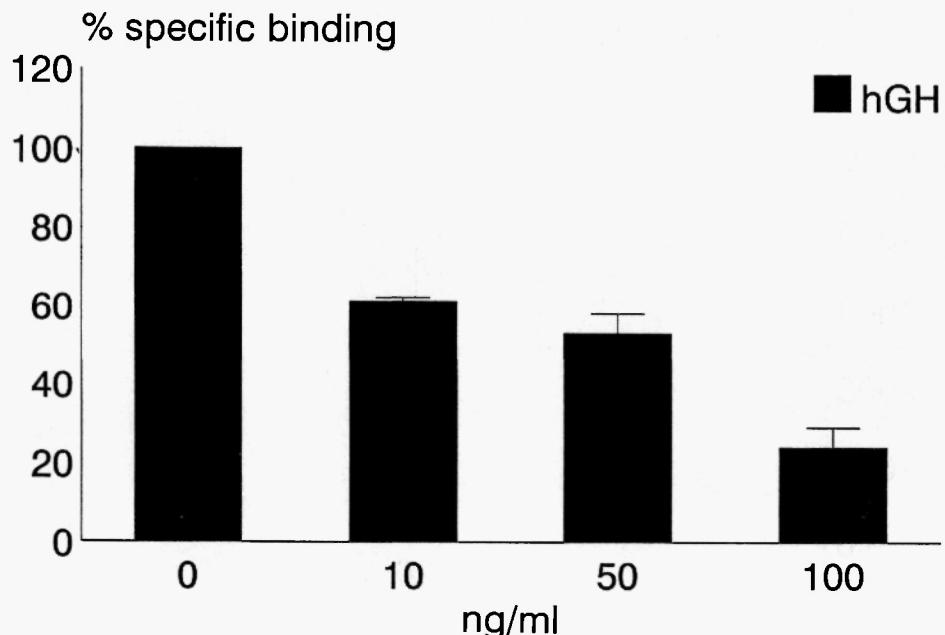
The above-mentioned results show that transformed porcine cells of unknown renal sublocalization possess GH-R but prove neither that healthy nephrons possess the receptors, nor that GH-R are located in the proximal tubule of the nephron. To investigate this, isolated proximal tubules from healthy rabbits were incubated in the presence of increasing concentrations of  $^{125}\text{I}$ -hGH with or without oxygen present in the medium (Fig. 4).  $^{125}\text{I}$ -hGH related radioactivity was found associated with the tubules in a concentration-dependent manner. The time course of the association is shown in Fig. 5, and revealed a continuously increasing amount of tubule-associated radioactivity, without any evidence for an initial lag phase (Fig. 5). In both types of experiment (Figs. 4 and 5), tubule-associated radioactivity values in preparations incubated in the presence or absence of oxygen ( $\text{N}_2$  bubbling) were similar, indicating that the measured association did not need energy in



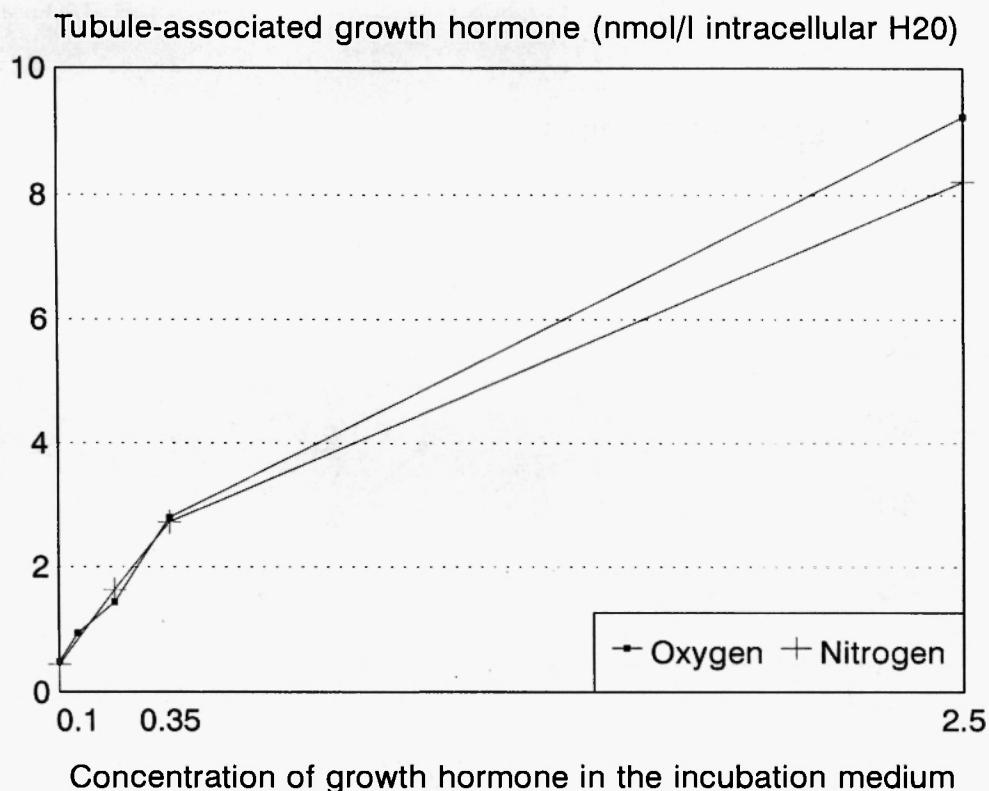
**Fig. 1:** Concentration-dependent binding of  $^{125}\text{I}$ -labelled human growth hormone to receptors on the porcine renal epithelial cell line, LLC-PK1. Means and SE of 3 experiments are presented.



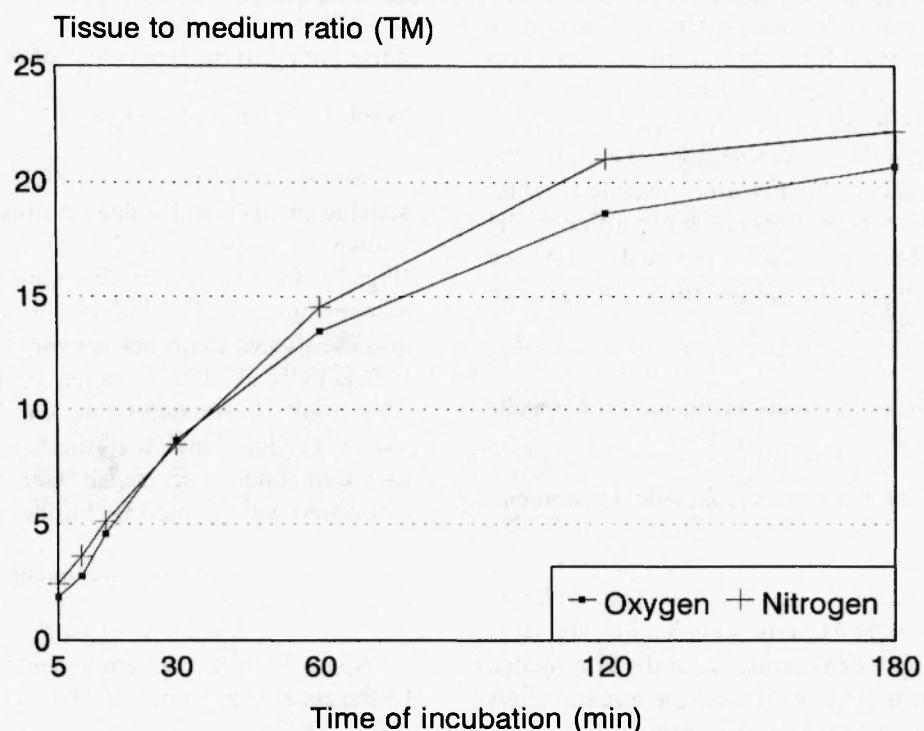
**Fig. 2:** Displacement of the binding of  $^{125}\text{I}$ -labelled human growth hormone and IGF-1 from receptors present on the porcine renal epithelial cell line, LLC-PK1. Means and SE of 4 experiments are presented.



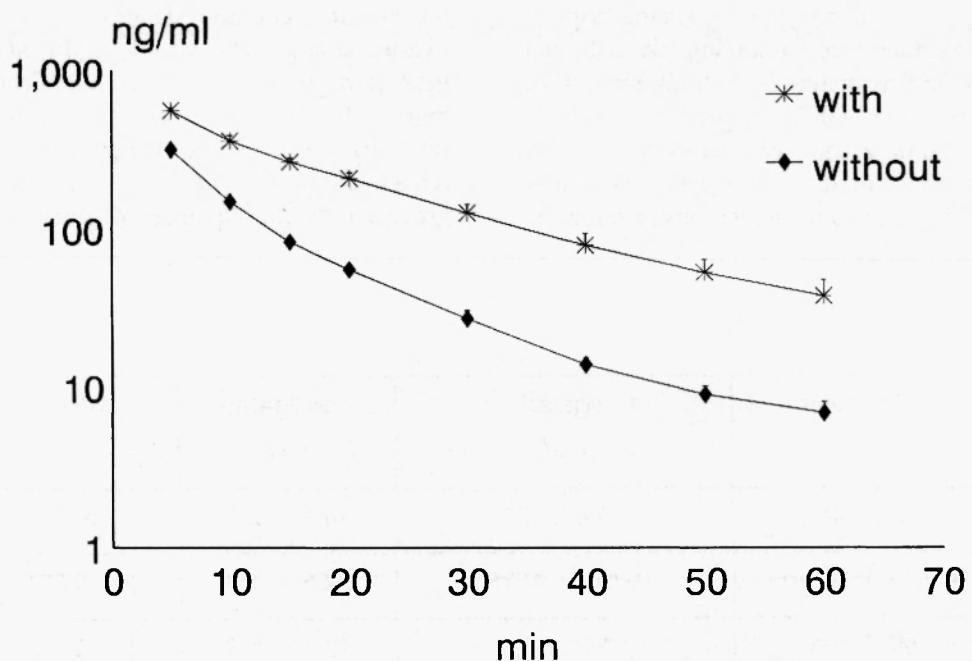
**Fig. 3:** Down-regulation induced by a 4 h preincubation period with increasing concentration of unlabelled hGH of the binding of  $^{125}\text{I}$ -labelled human growth hormone to receptors on the porcine renal epithelial cell line, LLC-PK1. Means and SE of 3 experiments are presented.



**Fig. 4:** Concentration-dependent association of growth hormone-related radioactivity with isolated, purified proximal tubules from healthy rabbits. A representative experiment from a series ( $n=5$ ) is shown.



**Fig. 5:** Time-dependent association of growth hormone-related radioactivity with isolated, purified proximal tubules from healthy rabbits. A representative experiment from a series ( $n=5$ ) is shown.



**Fig. 6:** Plasma concentrations of hGH in intravenously dosed rats with (x) and without (◆) renal ligation following administration of  $50 \mu\text{g}$  hGH/kg (mean  $\pm$  SE,  $n=6$ ).

order to take place. In the presence of excessive amounts ( $10 \mu\text{M}$ ) of unlabelled hGH, complete abolition of the binding of  $^{125}\text{I}$ -hGH to the rabbit tubules was observed (data not shown), implying that the binding was displaceable. Overall, the experiments indicate that freshly isolated rabbit proximal tubular cells possess high affinity GH binding sites that are likely candidates for the receptor responsible for stimulating tubular cell function in the rabbit /17/.

#### Pharmacokinetic experiments in normal and renally ligated rats

At the end of the pharmacokinetic experiment, rats with and without renal vessel ligation had serum creatinine values of  $104 \pm 8.4$  and  $73.5 \pm 5.3 \mu\text{mol/l}$ , respectively ( $p < 0.02$ ). The plasma concentrations of hGH were significantly higher in the ligated group when compared to the non-ligated group; see Fig. 6. The pharmacokinetic parameters obtained by non-compartmental analysis are shown in Table 1. The difference in GH clearance between the intact rats and the vessel-ligated rats exceeded that predicted from a decrease of GFR to zero since we have found GFR in Sprague-Dawley rats of this age to be approx.  $8 \text{ ml/min per kg}$  (manuscript in preparation). Furthermore, assuming an unbound GH fraction of approximately  $0.75$ ,  $6 \text{ ml/min per kg}$  (fraction unbound  $\times$  GFR) appears a realistic estimate of filtration-associated GH clearance. An active process, i.e. peritubular uptake via renal receptors for GH, such as those demonstrated in the

preceding sections, must consequently be contributing to the renal elimination of GH in rats.

#### Whole body autoradiography

Animals treated with  $^{125}\text{I}$ -hGH exhibited intense staining in liver and kidney cortex, i.e. the tissues known to possess the largest quantity of GH-R (Fig. 7). In accordance with a previous study /15/, administration of  $^{125}\text{I}$ -iodide did not cause staining in these tissues (data not shown), and the observed radioactivity is thus assumed to be drug related. This result substantiates that GH-R are present in kidney cortices, and that these receptors mediate the component of renal GH elimination not associated with glomerular filtration.

#### Effects of long-term treatment with GH on electrolytes in rats

Apart from the apparent contribution of GH-R to the renal elimination of GH, these receptors are considered *inter alia* to mediate the acute anti-saluretic effects of GH /10/. Since GH-associated fluid retention and edema formation is transient and with a rapid onset, we investigated whether the anti-saluretic effect of GH had diminished due to homeostatic counterregulation after treatment of ovariectomized rats with  $1 \text{ mg/kg}$  hGH for half a year. In absolute terms, hGH significantly increased kidney weight after 6 months treatment, but the ratio between kidney weight and body weight remained constant at 0.03, reflecting that kidney growth is a consequence of global anabolism. As

**TABLE 1**  
Pharmacokinetic parameters in rats with and without renal ligation following i.v. administration of  $50 \mu\text{g}$  hGH/kg  
(mean  $\pm$  SE)

Parameter	ligated $n=6$	non-ligated $n=6$	P
T $\frac{1}{2}$ min	$15.6 \pm 1.7$	$14.3 \pm 2.0$	NS
AUC min*ng/ml	$9898 \pm 1278$	$3065 \pm 169$	$P < 0.001$
MRT min	$25.3 \pm 2.5$	$19.1 \pm 1.3$	$P < 0.05$
CL ml/min/kg	$5.4 \pm 0.5$	$16.6 \pm 0.9$	$P < 0.001$



**Fig. 7:** Representative (n=3) whole body autoradiograph of a rat 30 min after dosing with  $^{125}\text{I}$ -hGH. Staining of the liver and kidney regions is evident.

shown in Table 2, normalized clearance estimates were similar before and after chronic dosing of 1 mg/kg hGH even though there was a trend toward reduced phosphate clearance at the high dose. Analysis of serum electrolytes revealed no change in sodium and potassium levels, whereas serum phosphate increased significantly ( $p<0.05$ ) and in a dose-dependent manner:  $1.73 \pm 0.08$  mM (placebo),  $1.81 \pm 0.04$  mM (0.05 mg/kg hGH) and  $2.29 \pm 0.08$  mM (1 mg/kg hGH). Urine volumes were not affected by the treatment.

#### Plasma renin activity (PRA) in rats treated with GH for one week

In order for plasma renin activity (PRA) to be significantly involved in GH-induced electrolyte retention in rats, a time relationship between the antialuresis (onset within a few hours) and PRA must exist. We consequently conducted a one week study with daily administration of 2 mg/kg hGH s.c. to rats, and measurement of PRA immediately before each GH dose. At the initiation of the study, PRA was approx. 3 ng/ml per h. This value did not change significantly until the fifth day of hGH dosing (Fig. 8). Consequently, it appears unlikely that a change in PRA contributes significantly to the rapid effects of GH on fluid homeostasis in rats.

#### DISCUSSION

By use of binding assays with  $^{125}\text{I}$ -labelled ligands, the present study has clearly demonstrated that the kidney possesses high affinity receptors for GH. The binding characteristics and modulation of these GH-R appear similar to the classical GH-R, present in, e.g., the human IM-9 cell line /18/. The combined findings in the experiments with a porcine renal cell line, isolated rabbit proximal tubules, and autoradiography of rats, indicate that classical GH-R are present in the proximal tubules of the renal cortex. Furthermore, the i.v. study in rats with surgical ligation of the kidney vessels demonstrated that the kidneys are involved in elimination of GH not only via glomerular filtration, but also by peritubular uptake and degradation of the hormone. We suggest that this process is related to occupancy of GH-R by GH - reaching receptors residing in the basolateral membrane via the circulation - with subsequent cell stimulation and, ultimately, internalization of the receptor-ligand complex and degradation of the hormone. The lack of internalization of the receptor-ligand complex in the present rabbit tubule preparations is probably caused by the low incubation temperature (25°C), resulting in very slow internalization kinetics. The participation of peritubular GH-R in the renal clearance of GH is not yet resolved /9,19-21/, but

TABLE 2

Effect of chronic growth hormone treatment of ovariectomized rats on renal clearance ( $\mu\text{l}/\text{min}$  per kg body weight). Mean values and standard deviations in groups of ten animals are shown.

Treatment	Sodium clearance	Phosphate clearance	Creatinine clearance
placebo treatment	11 $\pm$ 4	639 $\pm$ 249	3005 $\pm$ 786
low dose GH: 50 $\mu\text{g}/\text{kg}$	10 $\pm$ 5	576 $\pm$ 191	2942 $\pm$ 901
high dose GH: 1 mg/kg	17 $\pm$ 7	554 $\pm$ 235	2938 $\pm$ 1170

Plasma renin activity (ng/ml per h)

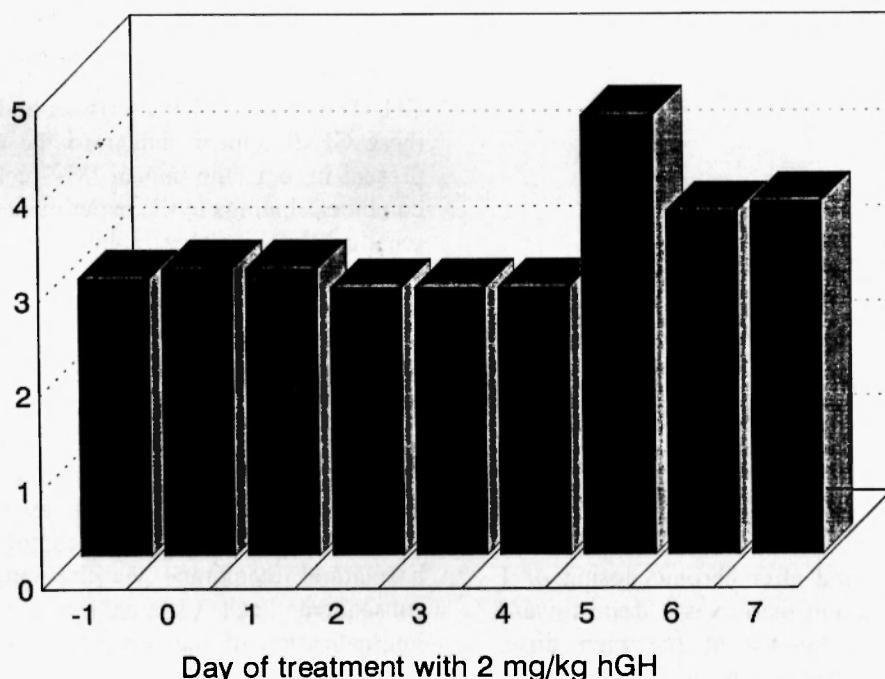


Fig. 8: Plasma renin activity (PRA) in groups (n=10) of rats dosed daily with 2 mg/kg hGH. At day 5, a significant increase in PRA was measured. The PRA determination on day 0 was made immediately before the first hGH dose.

the present *in vitro* data indicate that dynamic GH-R are present at a relevant site within the nephron.

In the kidney, GH directly stimulates - perhaps by activation of the phospholipase C pathway /6/ - gluconeogenesis /22/ and ammoniagenesis /23/. All

other effects of GH on the kidney are believed to be mediated by the classical hepatic IGF-1 response to GH or via induction of local IGF-1 gene expression /24/. In the latter case, a GH/IGF-1 axis acting in an auto-/paracrine manner is putatively responsible for

kidney responses. *In vivo*, the notion that GH acts on kidney tissue indirectly via IGF-1 has generally been confirmed in rat studies /25-27/, and experiments have also demonstrated the importance of IGF-1 in renal regulation by GH. In the GH-deficient *dw/dw* dwarf rat, a decrease in renal size and IGF-1 content occurs and can be reversed by GH or IGF-1 treatment /28/. In humans, the effects of GH on kidney filtration are not immediately detectable /29,30/, but after treatment for one week an increase in GFR is apparent /31/. The ligand binding experiments in the present study using a porcine renal epithelial cell line suggest, but do not prove, that GH-induced IGF-1 from tubular cells may participate in the renal functional activation seen following administration of GH to normal, but not uremic rats /27/. However, before extrapolation from animals to man is attempted, it should be noted that species dissimilarities may occur. For instance, an important difference in kidney pathophysiology between humans and rodents is that in human hypsomatotropinemia kidney morphology is normal, whereas a prolonged excess of GH in rodents leads to clear-cut glomerulosclerosis, as seen in GH-treated rats and GH transgenic mice /32,33/.

From a pharmacokinetic viewpoint, the present study clearly shows that the kidneys play a significant role in GH elimination from the rat body. The difference in clearance between ligated and non-ligated animals exceeded GFR in the rat, suggesting that hGH is eliminated by glomerular filtration accompanied by receptor-mediated clearance from the peritubular side of the nephron. The renal clearance accounted for approx. 2/3 of the total clearance. The results are in accordance with a study in rats showing that the kidney is responsible for about 70% of GH turnover /5/. Even though blood pressure was consistently lower in ligated animals it is believed that the influence on systemic circulation could not account for the observed difference in clearance. Moreover, the metabolic state of the ligated animals was only moderately affected during the study. The notion that the renal elimination of hGH appeared to contribute by more than could be attributed to glomerular filtration emphasizes that internalization and degradation of receptor-bound GH in the kidney cortex may be responsible for a proportion

of the renal clearance of GH. Receptor-bound GH in the kidney cortex probably accounted for a significant part of the autoradiographic staining in the WBA experiment. Receptor-mediated hormone clearance, as suggested here, has been postulated for insulin /34/.

The only electrolyte-related change that remained evident following a treatment period of half a year in the present rat study, was an elevation of serum phosphate. The role of the renin-angiotensin system (RAS) in the electrolyte-associated fluid retention seen during the early phase of human GH treatment remains elusive for some /12/ but not all /35/ authors. In the present report, RAS activation was not prominent, since we were unable to detect any increase in PRA during the first 4 days of GH administration. This is in line with a recent study in dwarf rats /36/ in which PRA only increased after prolonged GH treatment. Thus, in rats PRA does not appear to mediate the anti-saluretic and fluid-retaining effects of GH that occur within a few hours after a single GH dose /10/. We cannot, however, exclude the possibility that, e.g., upregulation of the renal angiotensin II receptor may participate in the phenomenon.

In conclusion, the data presented here support the existence of important mutual interactions between GH and the healthy kidney, viz. the receptor-mediated effects of GH on the kidney that, in turn, contribute significantly to GH elimination by glomerular filtration and probably receptor-mediated clearance of the hormone.

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