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Growth hormone receptor abundance in tibial growth plates of uremic rats: GH/IGF-I treatment

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Background. Children with chronic renal failure (CRF) exhibit growth retardation and a disturbed growth hormone/insulin-like growth factor-I (GH/IGF-I) axis. Treatment of children with CRF with GH or GH/IGF-I can partially restore linear growth. The molecular basis for decreased longitudinal growth is not known but may involve an impaired action of GH.

Methods. We used the growth-retarded uremic rat model to determine the abundance and distribution of GH receptors (GHRs) in the tibial epiphyseal growth plate and the influence of GH, IGF-I, or combined GH/IGF-I treatment. Pair-fed rats were used as the control.

Results. While all treatment regimes increased body length and weight in both rat groups, only GH/IGF-I treatment increased the total growth plate width. This involved an increase in cell number in the hypertrophic zone, which could also be induced by IGF-I alone. Immunohistochemical analysis showed that uremic rats had decreased abundance of GHRs in the proliferative zone, and only GH/IGF-I therapy could overcome this decrease. These data thus suggest that growth retardation in uremic rats is, at least in part, due to a decrease in GHR abundance in chondrocytes of the proliferative zone of the tibial growth plate. This decreased GHR abundance can be overcome by combined GH/IGF-I therapy, thus enhancing generation and proliferation of hypertrophic zone chondrocytes and increasing growth-plate width.

Conclusion. These studies point to a mechanism for the growth retardation seen in children with CRF, and suggest that combined GH/IGF-I treatment may provide more effective therapy for these patients than GH alone.

Chronic renal failure (CRF) in children is associated with severe growth failure. Recent studies have shown that a perturbed growth hormone/insulin-like growth factor-I (GH/IGF-I) axis is one of many factors that may contribute to this growth retardation. In particular, CRF is associated with normal or elevated serum levels of GH and normal or decreased serum levels of IGF-I depending on the state of renal failure, suggesting a state of GH insensitivity in target organs [1–6]. Treatment of CRF children with recombinant human GH (rhGH) can partially restore linear growth, suggesting that the insensitivity to endogenous GH can be overcome [7–11]. Rats with experimental uremia are also growth retarded, and studies have shown varying degrees of restoration of linear growth with GH [12–20], IGF-I, or combinations of GH/IGF-I treatment [21–23].

The molecular basis for the decrease in longitudinal growth seen in CRF is not clearly understood. GH is a major regulator of bone growth [24–27]. GH is likely to act directly on GH receptors (GHRs) on growth-plate chondrocytes [28] and, in association with local production of IGF-I, increases growth plate width [29]. Uremic rats show a disorganized tibial epiphyseal growth plate, which may be slightly wider or narrower than normal controls [13, 22]. Treatment with GH results in expansion of the growth plate width, which may selectively involve specific zones [17, 23]. We therefore hypothesized that retarded linear bone growth in uremia may be due to a change in GHR distribution and/or abundance in the growth plate. Furthermore, we predicted that treatment with GH or IGF-I or combined GH/IGF-I may modulate receptor abundance, thus providing a potential mechanism for growth-promoting effects. In this study, we have used the uremic rat model to characterize, by immunohistochemistry, the distribution and abundance of GHRs in the tibial epiphyseal growth plate. We have then examined the effect of GH, IGF-I, and GH/IGF-I therapy on abundance and distribution of GHRs in the tibial growth plate of the uremic rat.

METHODS

Female Sprague-Dawley rats (Ivanovas Co., Kisslegg/Allgäu, Germany) weighing 70 to 80 g were used for the

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experiments. One week prior to the study, the animals were kept in single cages at constant room temperature (24°C) and humidity (70%) on a 12-hour on/off light cycle. The animals had free access to food (Altro min C 1000 diet; Altromin Co., Lage/Lippe, Germany) and deionized water. The diet contained 13800 kJ/kg, 0.95% calcium, 0.8% phosphorus, 500 IU/kg vitamin D_3, and 18% protein (wt/wt). The animals were randomized to the experimental groups according to their body weight. The animals were subjected to two-stage subtotal nephrectomy (NX), as described previously [13, 30]. Five days after subtotal NX of the left kidney, when the animals had a mean body weight of 132 g, the contralateral kidney was removed, and uremia resulted. Control animals were sham operated (renal decapsulation). Uremic animals had free access to food and water, whereas the control groups were pair fed to the uremic group, as described previously [21]. Pair feeding started after the first stage of operation. The duration of uremia in all experiments was 10 days.

Experimental protocol

The uremic animals and the pair-fed control animals were randomized into four groups ($N = 4$ animals/treatment group). Group 1 received vehicle twice daily subcutaneously. Group 2 was treated with 7.5 IU rhGH/kg/day twice daily subcutaneously. Group 3 was treated with 3 mg rhIGF-I/kg/day twice daily subcutaneously, and group 4 received a combination of rhGH and rhIGF-I in the doses indicated previously in this article. Recombinant human GH (Genotropin) and rhIGF-I (CH/B/60229-51) was a gift of Pharmacia and Upjohn Company (Stockholm, Sweden). Hormonal and vehicle injection were started after the second operation for subtotal NX. The duration of treatment was 10 days.

Processing of tissue

Tibias were excised, broken midshaft, trimmed of excess tissue, and placed in placed in 10% formalin in phosphate-buffered saline (PBS; Oxiod, Basingstoke, Hampshire, UK) for 24 hours at 4°C. Tibias were then decalcified in 5% formic acid at 4°C for up to 20 days. Following decalcification, tibias were split in the midline sagittal plane and processed through graded ethanol, cleared in two changes of xylene, and infiltrated with paraffin wax. Six micrometer sections were cut, mounted on aminoalkylsilane-coated slides [31], and baked overnight at 37°C.

Immunohistochemistry

Epiphyseal growth plate sections were subjected to immunohistochemistry as previously described [32] with modifications as follows: Monoclonal antibody 263 (mAb 263) [33] was used at a concentration of 1:100 to detect GHR. Monoclonal antibody 7 (mAb 7) [33] was used as a negative control (1:100). The Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA, USA) was used for to detect bound primary antibody. Briefly, sections were dewaxed in Histolene (Riverstone, New South Wales, Australia), rehydrated by passage through graded ethanol and ultrapure water (Liquipure Continental Water System, Melbourne, Victoria, Australia), and incubated in H_2O_2 in PBS (pH 7.4) to quench endogenous peroxidase activity, and nonspecific protein binding was eliminated by incubation with horse serum. Sections were then incubated with (*1*) diluted primary antibody (mAb 263, mAb 7), followed by (*2*) diluted biotinylated second antibody (antimouse), and then (*3*) Vectastain ABC reagent. Following the diaminobenzidine (DAB)/peroxidase reaction, sections were counterstained with hematoxylin and eosin, dehydrated in graded ethanol, cleared in histolene (Riverstone), and mounted under DPX (BDH, Dorset, UK).

Analysis

In vivo measurement of body length and weight. Body length and weight were measured during the afternoon in nonfasting animals. Nose-to-tail tip distances were measured in anesthetized animals under complete muscle relaxation as described previously [13, 30]. The increase in body weight and length in both uremic and pair-fed animals was used for analysis.

Growth plate width analysis. Total growth plate and regional [proliferative (P) and hypertrophic (H)] growth plate width were analyzed using the microcomputer imaging device (MCID) M4 image analyzer (Imaging Research Inc., St. Catherines, Canada). Total growth-plate width was measured as the distance from the bone matrix in the reserve zone to bone matrix in between the zone of calcifying cartilage and developing trabeculae of the metaphysis. P zone width was taken as the distance from the bone matrix in the reserve zone to the distal border of the last line of P cells prior to clear H change. H zone width was taken as the distance from this line to the mature edge of the growth plate. Following calibration of the MCID system, 12 different measurements (μm) for each growth plate or growth plate zone were taken, and a mean width was calculated.

Cell number. Cell numbers within the P and H zones of tibial epiphyseal growth plates were counted blind from three different microscopic fields per growth plate, using a grid to maintain standard area counted. An average of the three fields was calculated, and data were then expressed as average cell numbers for each treatment group.

Growth hormone receptor-positive chondrocyte identification and distribution analysis. GHR-positive chondrocytes of tibial epiphyseal growth plates, indicated by a dark-brown peroxidase reaction, were counted by a blinded observer in both the P or H zones. Three differ-

ent microscopic fields per growth plate were assessed for GHR-positive cells using a grid to maintain a standard area, which was analyzed. Data were expressed as the percentage of cells that were GHR positive per the total number of cells per zone.

Statistical analysis

Data are reported as mean ± SD. For comparison of length gain and weight gain between groups, a two-way analysis of variance (ANOVA) was performed followed by all pair-wise multiple comparison (Student–Newman–Keuls method). Growth plate measurements were analyzed by a one-way ANOVA followed by a Newman–Kuels Multiple Comparison test. $P < 0.05$ was accepted as being statistically significant. All analyses were undertaken using Prism GraphPad software (San Diego, CA, USA).

RESULTS

Surrogate markers for renal function measured at sacrifice indicated a significant impairment of glomerular filtration rate. Serum creatinine values were (mean and range) 1.1 (0.7 to 1.3) mg/dL in uremic animals and 0.3 (0.2 to 0.3) mg/dL in pair-fed controls. GH and IGF-I did not significantly influence those values.

GH, IGF-I, or GH/IGF-I treatment increases the weight and length of uremic and pair-fed rats

Figure 1A represents the analysis of total weight gain of both uremic and pair-fed rat groups treated with either vehicle (saline), GH, IGF-I, or combined GH/IGF-I. The administration of GH, IGF-I, or GH/IGF-I to uremic rats resulted in a significant weight gain ($P < 0.001$), with GH or GH/IGF-I being more effective than IGF-I ($P < 0.01$). No additive effect of GH and IGF-I in combination was seen in uremic animals compared with GH alone. In the case of pair-fed rats, only GH was effective alone ($P < 0.001$), but an additive effect of the combination of GH/IGF-I was seen ($P < 0.001$). The weight gain for uremic rats either saline treated, IGF-I treated, or GH/IGF-I treated was significantly different to their equivalent pair-fed group ($P < 0.001$, $P < 0.05$, $P < 0.01$, respectively). In contrast, GH-treated uremic rats gained weight to the same extent as GH-treated pair-fed rats.

Figure 1B summarizes the analysis of body length gain of both uremic and pair-fed rat groups treated with either vehicle, GH, IGF-I, or GH/IGF-I. Treatment of both uremic and pair-fed rats with GH, IGF-I, or combined GH/IGF-I resulted in a significant gain in body length when compared with their respective control groups ($P < 0.001$). However, no difference was seen between the effect of GH or IGF-I alone or in combination in either uremic or pair-fed animals. Uremic rats, including those

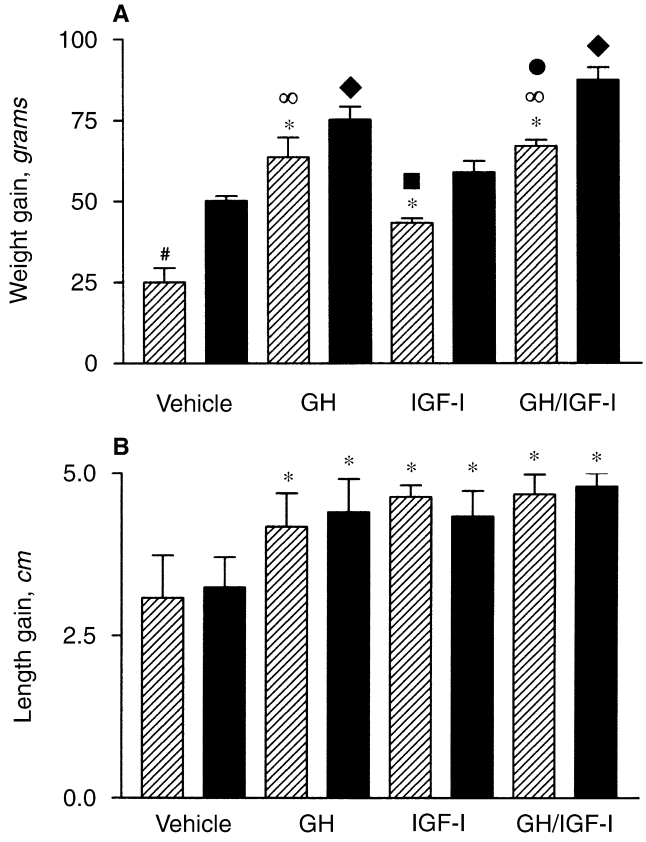

Fig. 1. Effect of growth hormone (GH), insulin-like growth factor-I (IGF-I), or combined GH/IGF-I treatment for 12 days on weight gain and length gain in uremic (▨) or pair-fed (■) rats. (*A*) Weight gain in uremic or pair-fed rats treated with vehicle, IGF-I, GH, or GH/IGF-I. $^{*}P < 0.001$ vs. vehicle-treated uremic rats; $^{\infty}P < 0.01$ vs. IGF-I–treated uremic rats; $^{\blacklozenge}P < 0.001$ vs. pair-fed vehicle-treated rats; $^{\#}P < 0.001$ vs. vehicle-treated pair-fed rats; $^{\blacksquare}P < 0.05$ vs. IGF-I treated pair-fed rats, $^{\bullet}P < 0.01$ vs. GH/IGF-I treated pair-fed rats. (*B*) Length gain in uremic (▨) or pair-fed (■) rats treated with vehicle, IGF-I, GH, or GH/IGF-I. $^{*}P < 0.001$ compared with respective control groups, that is, uremic/vehicle-treated or pair-fed/vehicle-treated rats. Data are mean ± SD.

treated as previously described, were not significantly different in their body length gain when compared with their respective pair-fed, treated rat group.

Cumulative food intake for the uremic rats, and thus pair-fed rats, was 142 ± 12 g, and this volume of food intake did not significantly alter with hormonal treatment.

Tibial epiphyseal growth-plate widths are increased by combined GH/IGF-I treatment

The total width of tibial epiphyseal growth plates from uremic rats was not significantly different to pair-fed rats (Fig. 2A). Only GH/IGF-I treatment of both uremic or pair-fed rats resulted in a significant increase in total growth plate width when compared with their corresponding saline treated rat groups ($P < 0.01$, $P < 0.001$, respectively), but growth-plate width of uremic GH/IGF-

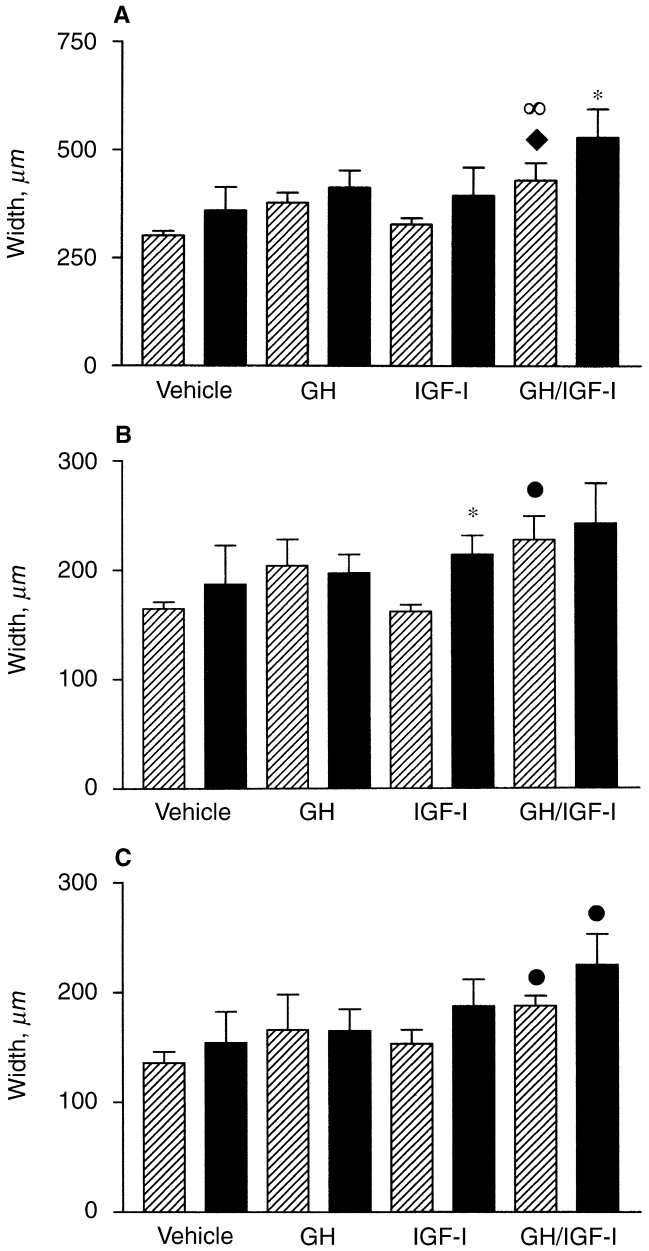

Fig. 2. GH/IGF-I treatment of uremic (▨) and pair-fed (■) rats increases total growth-plate width. (*A*) Total growth-plate width is increased by GH/IGF-I treatment of uremic and pair-fed rats. $^{\blacklozenge}P < 0.01$ vs. uremic vehicle-treated rats; $^{*}P < 0.001$ vs. pair-fed vehicle-treated rats; $^{\infty}P < 0.01$ vs. GH/IGF-I-treated pair-fed rats. (*B*) The P zone width of tibial epiphyseal growth plates of uremic rats is increased by GH/IGF-I treatment. $^{\bullet}P < 0.05$ vs. vehicle-treated uremic rats; $^{*}P < 0.05$ vs. IGF-I-treated uremic rats. (*C*) The H zone width of tibial epiphyseal growth plates of uremic rats is increased by GH/IGF-I treatment. $^{\bullet}P < 0.05$ vs. vehicle-treated uremic or pair-fed rats, respectively. Data are mean ± SD.

I–treated rats was significantly less than pair-fed GH/IGF-I–treated uremic rats ($P < 0.01$).

Tibial growth-plate widths were further analyzed by assessing regional widths, within the P (Fig. 2B) or H zones (Fig. 2C), of each rat treatment group. Only uremic rats treated with GH/IGF-I showed an increase in P zone width when compared with untreated uremic rats ($P < 0.05$). No differences in P zone widths were seen within the pair-fed rat groups under any treatment regime compared with saline-treated, pair-fed rats (Fig. 2B), although the P zone width of IGF-I–treated pair-fed rats was significantly wider compared with IGF-I–treated uremic rats ($P < 0.05$).

Hypertrophic zone widths of tibial growth plates were increased following GH/IGF-I therapy for both uremic and pair-fed rats when compared to uremic-or pair-fed vehicle-treated rats, respectively ($P < 0.05$; Fig. 2C). In contrast, there was no difference in the H zone width between uremic and pair-fed groups for all treatment regimes.

GH/IGF-I treatment increases chondrocyte cell numbers of the H zone in tibial epiphyseal growth plates of both uremic and pair-fed rats

Chondrocyte cell numbers within both P and H zones of tibial growth plates from all experimental rat groups were analyzed (Fig. 3). Uremic rats under any treatment regime did not show a significant change in P zone chondrocyte numbers. In contrast, only GH treatment of pair-fed rats resulted in a significant increase in P zone chondrocyte number when compared with GH/IGF-I treatment of pair-fed rats ($P < 0.05$). A comparison of uremic rats with pair-fed rats under the same treatment regime did not reveal any significant differences in P zone chondrocyte number.

In uremic rats, either IGF-I or GH/IGF-I treatment led to an increase in H cell numbers of the tibial growth plate when compared with vehicle-treated uremic rats ($P < 0.001$ and $P < 0.01$, respectively; Fig. 3B). IGF-treated uremic rats had significantly more H cells when compared with both GH-treated and GH/IGF-I–treated uremic rats ($P < 0.001$). Furthermore, IGF-I–treated uremic rats had significantly more H cells when compared with pair-fed rats treated with vehicle, GH, IGF-I, or GH/IGF-I ($P < 0.001$). Only GH/IGF-I treatment of pair-fed rats caused an increase in H chondrocyte number when compared with vehicle-treated pair-fed rats ($P < 0.05$).

GHR abundance in tibial epiphyseal growth plates is decreased in uremia, but increased by GH/IGF-I treatment

Growth hormone receptor abundance in tibial epiphyseal growth plate chondrocytes was assessed by immunohistochemical staining [Fig. 4A (i), (ii)], and the results are expressed as the percentage of cells that are GHR positive. Firstly, tibial growth plate GHR abundance in uremic rats was decreased when compared with growth plates from pair-fed rats ($P < 0.05$; Fig. 4B). This decrease in GHR abundance was seen in the P zone only

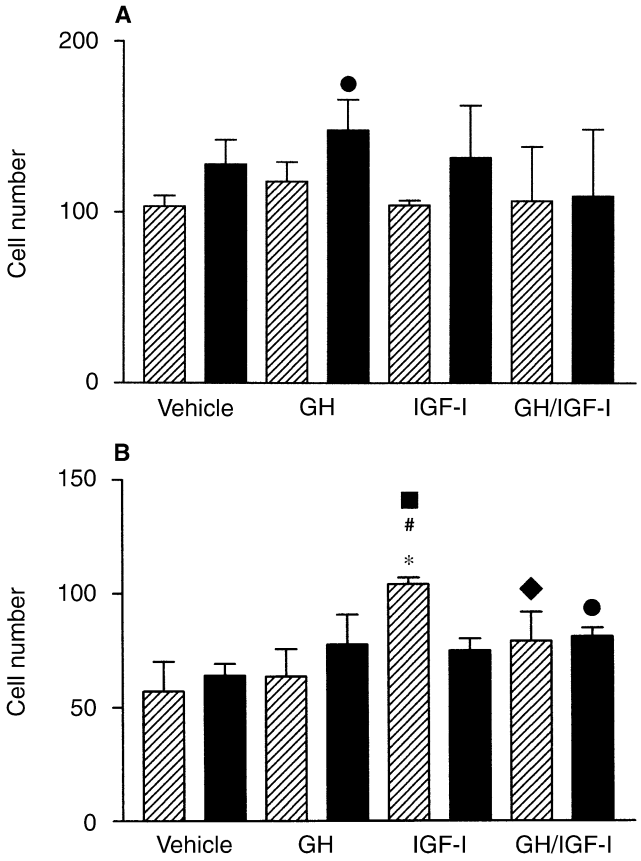

Fig. 3. GH/IGF-I treatment of uremic (▨) or pair-fed (■) rats increases H cell number of tibial epiphyseal growth plates. (*A*) Analysis of P zone chondrocyte cell number following treatment with vehicle, GH, IGF-I, or GH/IGF-I. Only GH treatment of pair-fed rats results in an increase in P zone chondrocytes. $^{\bullet}P < 0.05$ vs. GH/IGF-I-treated pair-fed rats. (*B*) Analysis of H zone chondrocyte cell number following treatment with vehicle, GH, IGF-I, or GH/IGF-I. $^{\blacklozenge}P < 0.01$ vs. vehicle-treated uremic rats; $^{\bullet}P < 0.05$ vs. vehicle-treated pair-fed rats; $^{*}P < 0.001$ vs. vehicle-treated uremic rats; $\#P < 0.001$ vs. GH-treated and GH/IGF-I-treated uremic rats; $^{\blacksquare}P < 0.001$ vs. vehicle-, GH-, IGF-I–, and GH/IGF-I–treated pair-fed rats. Data are mean ± SD.

and was not overcome by individual GH or IGF-I treatment of uremic rats. In contrast, the GHR abundance in the P zone of GH/IGF-I–treated uremic rats was significantly increased when compared with uremic vehicle-treated rats ($P < 0.001$). GH/IGF-I therapy of uremic rats was able to restore GHR abundance in the P zone to levels seen in vehicle-, GH-, IGF-I–, or GH/IGF-treated pair-fed rats. GH- or IGF-I–treated uremic rats also had reduced GHR abundance in P zone chondrocytes when compared with their corresponding pair-fed treated rats ($P < 0.01$, $P < 0.05$, respectively). GH treatment of pair-fed rats also led to an increase in GHR abundance in the P zone when compared with vehicle-treated pair-fed rats ($P < 0.05$).

Growth hormone receptor abundance within the H region of tibial growth plates was not significantly affected by any treatment regime for either uremic or pair-fed rat groups (Fig. 4C).

DISCUSSION

We have shown in this study that combined GH and IGF-I therapy overcomes the growth failure of uremia, and that this involves expansion of growth plate width and cell number in the H zone. While such findings have been described previously by ourselves and others, the present study provides a likely mechanism for these changes. To our knowledge, we demonstrate for the first time that uremic rats show a decreased abundance of GHR-positive chondrocytes in the P zone of the tibial epiphyseal growth plate. Furthermore, and most significantly, we show that combined therapy with GH and IGF-I in these uremic rats results in an increased abundance of GHRs in the P zone of the growth plate, thus reversing the adverse effects of uremia on GH-responsive chondrocytes and leading to growth plate expansion in response to combined GH and IGF-I.

The reduction in GHR abundance in P zone chondrocytes in uremia is clearly a specific effect of uremia, since it is not seen in pair-fed animals with a similarly reduced food intake. Uremia has been previously shown to be associated with reduced GH binding to liver membranes [34] and associated reduction in hepatic GHR mRNA expression [35]. Although we did not examine GHR mRNA in the present study, it is likely that this would be similarly reduced in the growth plate to account for reduced GHR protein abundance. The in vitro findings of Mak and Pak showing that chondrocytes from uremic rats show "end-organ resistance" to GH and IGF-I are consistent with reduced abundance of GHR and possibly insulin-like growth factor-I receptor (IGF-IR) [36].

Although there are significant data on the in vivo growth and weight-enhancing effects of GH and IGF-I alone or together in growth-retarded uremic rats [12–23], the underlying mechanisms of their effects have remained unclear. Our finding that the combination of GH and IGF-I is more effective on both weight gain and growth plate expansion than GH or IGF-I alone is consistent with findings of Hazel et al [22], who showed similar effects on weight and metabolic changes.

The interpretation of the growth data in our present study is, however, limited because the study was designed for a semiquantitative analysis of GHR expression. In contrast to earlier experiments [21, 23], the duration of the experiment was reduced (but long enough to regulate the GHR by hormonal treatment). The treatment dose of GH and of IGF-I was suboptimal, and the number of animals per group was smaller. Our earlier experiment with maximally effective doses of GH and of IGF-I demonstrated an additive effect of GH and of IGF-I [23]. In the present study, we used smaller doses of GH and

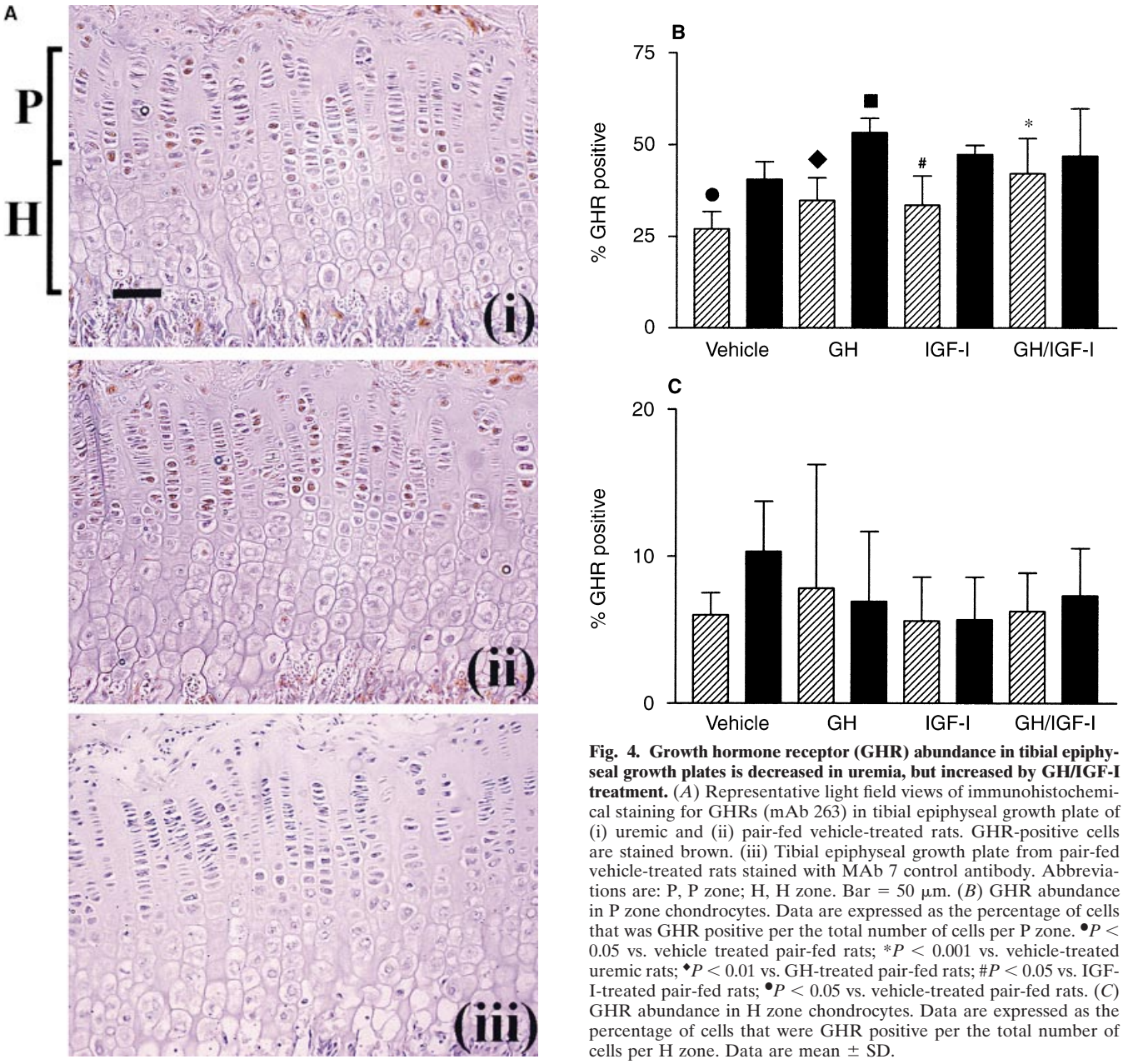

Fig. 4. Growth hormone receptor (GHR) abundance in tibial epiphyseal growth plates is decreased in uremia, but increased by GH/IGF-I treatment. (*A*) Representative light field views of immunohistochemical staining for GHRs (mAb 263) in tibial epiphyseal growth plate of (i) uremic and (ii) pair-fed vehicle-treated rats. GHR-positive cells are stained brown. (iii) Tibial epiphyseal growth plate from pair-fed vehicle-treated rats stained with MAb 7 control antibody. Abbreviations are: P, P zone; H, H zone. Bar = 50 μm. (*B*) GHR abundance in P zone chondrocytes. Data are expressed as the percentage of cells that was GHR positive per the total number of cells per P zone. $^{\bullet}P < 0.05$ vs. vehicle treated pair-fed rats; $^{*}P < 0.001$ vs. vehicle-treated uremic rats; $^{\blacklozenge}P < 0.01$ vs. GH-treated pair-fed rats; $\#P < 0.05$ vs. IGF-I-treated pair-fed rats; $^{\bullet}P < 0.05$ vs. vehicle-treated pair-fed rats. (*C*) GHR abundance in H zone chondrocytes. Data are expressed as the percentage of cells that were GHR positive per the total number of cells per H zone. Data are mean ± SD.

IGF-I, intending not to maximally up-regulate the GHR by either GH or IGF-I alone. Our results demonstrate that the combination of GH and IGF-I was more effective at enhancing GHR expression in the tibial growth plate than the treatment with one of the hormones alone. This is consistent with the earlier results [23]. However, we cannot exclude that a higher dose of GH or of IGF-I would also have had a greater effect on the GHR expression. For logistic reasons, we could not perform dose-response experiments.

The effect of GH/IGF-I to increase GHR abundance in uremic rats was consistent with its positive effects on growth plate width, nose-tail length, and weight gain. This provides further strong support for our original hypothesis that the mechanisms of action of these peptides on linear growth might be via regulation of growth plate GHRs. The initial finding that the growth failure of uremia was associated with reduced expression of tibial GHRs also, of course, pointed to this possibility. In contrast, pair-fed animals maintained higher levels of GHR abundance, showing no change in response to GH/IGF-I treatment, in spite of similar linear growth and weight responses. Furthermore, in pair-fed animals, increased growth plate width in response to GH and IGF-I

was limited to the H zones. In contrast, in uremic rats treated with GH and IGF-I, both P and H zones expanded, in concert with the increased GHR expression in the P zone. These findings clearly suggest that mechanisms of growth regulation via the GH/IGF-I axis in uremia are distinct from those in nutrition, specifically involving regulation of GH action via control of growth plate GHR abundance.

Our use of sham-operated pair-fed animals as controls for uremic animals clearly allowed us to distinguish the effects of uremia from the effects of inadequate nutrition per se. Uremia has a distinct effect on the metaphyseal width of long bones, particularly the H zone, an effect dependent on duration and severity of uremia [13, 23, 37]. Chondrocyte cell turnover per column is reduced, with a longer duration of the H phase and reduced cellular advance velocity and cartilage resorption, leading to an accumulation of cartilage cells at the H zone [37]. These processes are dependent on the severity of uremia, the duration of the experiments, and the age of the animals, explaining why differing findings on the height of the growth plate in uremia have been reported [13, 23, 37]. In the present experiments, serum creatinine and urea levels at sacrifice indicated a significant reduction of renal function in uremic animals compared with pair-fed controls. According to our model, uremia is even higher shortly after the second stage of subtotal NX. In earlier experiments, we observed within three days an increase of serum creatinine to about 1.6 mg/dL and of urea to more than 200 mg/dL [23]. As in earlier experiments, GH and IGF-I did not significantly influence renal function [23].

In our short-term experiments, the growth plate width did not differ between uremic and pair-fed control animals, probably because of the short duration of uremia. In contrast to our previous results [23], GH or IGF-I alone did not lead to increased growth plate width, probably because of the lower doses used and shorter experimental duration; they were, however, effective in combination. The increase of the H zone width following combined treatment with GH and IGF-I may be interpreted in the light of recent kinetic data [37]. Hormonal treatment might increase cell proliferation and also the conversion from proliferating cells into H cells, but not the resorption of H cells to the same degree.

The finding that GHR abundance was increased in the P and not the more mature H zone in response to GH/IGF-I is consistent with the original "dual effector" theory [38, 39], in which GH is responsible for differentiation of the less mature chondrocytes in the growth plate, in this case inducing further GHR expression and enhanced GH sensitivity. Our data showing an increase in growth plate width by GH/IGF-I treatment and the resultant increase in H zone chondrocyte number only further support the "dual effector" theory. Specifically, we suggest that an increase in GHR-responsive P zone chondrocytes would lead to an increase in the number of differentiated H zone chondrocytes. The findings of Hanna et al are also in support of these results, in which they described an increase in IGF-I messenger RNA in the P zone in response to GH therapy in uremic rats [17]. This again would be expected in response to direct local actions of GH and would be further enhanced by GHR up-regulation. It is once again consistent with the "dual effector" theory, in which proliferating chondrocytes synthesize IGF-I in response to GH and enhance H bone expansion. Consistent with this, IGF-I treatment of uremic rats increased H zone chondrocyte cell number, although it did not lead to an increase in growth plate cell width, suggesting that factors other than chondrocyte number, such as extracellular matrix production, contribute to growth plate width. An alternative model proposed by Hunziker, Wagner, and Zapf [40], whereby the role of GH and IGF-I overlap with respect to differentiation and proliferation, would also be consistent with our findings.

The dual role of GH and IGF-I in growth plate maturation and expansion is thus emphasized. It is not clear whether locally synthesized IGF can itself induce up-regulation of GHRs in vivo. Our earlier in vitro studies using cultured rat growth plate chondrocytes showed a positive effect of IGF-I on GHR abundance [41], while our recent study showed no effect [42]. In contrast, recent in vitro findings in bone cells suggest the converse [43]. Certainly, our current in vivo findings do raise the possibility that IGF-I may play a role in up-regulating the tibial GHR, since only GH/IGF-I, and not GH or IGF-I alone, significantly increase GHR expression in the uremic growth plate.

In spite of these findings that GHR expression and growth plate width are only increased by GH and IGF in combination, it is clear that both GH and IGF-I alone are able to increase overall linear growth and weight gain. In the case of IGF-I, we found that IGF-I therapy increases H zone chondrocyte numbers in uremic rats, consistent with the notion that expanded P zone clones are IGF-I responsive [38]. Clearly, there may be a number of other systemic or local mechanisms whereby GH or IGF-I alone increase linear growth and weight gain.

Our finding of decreased GHRs in rat tibial growth plates thus suggests that the growth retardation exhibited in children with CRF may be related to a similar reduction in GHR abundance, resulting in reduced sensitivity to endogenous GH. Consistent with this notion is the finding that children with CRF have low circulating levels of GH-binding protein, a marker for tissue GHR [44]. Up-regulation of P zone chondrocyte GHR expression in response to combined GH/IGF-I therapy is thus likely to enhance GH sensitivity and accounts for growth plate expansion, linear growth, and weight gain. Hence, these

studies not only suggest that combined GH/IGF-I treatment of CRF children may be a more appropriate therapy than GH or IGF-I alone, but also provide a mechanism for these observations.

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