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A single arginine residue determines species specificity of the human growth hormone receptor

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ABSTRACT **Although growth hormone (GH) receptors (GHRs) in many species bind human (h) GH as well as their own GH, the hGHR only binds primate GH. Arg^{43} in hGHR interacts with Asp^{171} of hGH. Nonprimates have a His in the position equivalent to residue 171 of primate GH and a Leu in position 43 of primate GHR. To determine whether Arg^{43} accounts for the species specificity of the hGHR, point mutations that changed Leu^{43} to Arg were introduced into the cDNAs encoding the bovine (b) GHR or the rat GH binding protein (GHBP) and these mutants or their wild-type (WT) counterparts were expressed in mouse L cells. Binding of hGH or bGH to transfected cells or to GHBP secreted into the incubation medium was assessed by displacement of ^{125}I-labeled hGH. WT and mutant bGHR bound hGH with similar affinity, but the affinity of the mutant receptors for bGH was reduced 200-fold. Likewise, WT and mutant GHBP bound hGH with equal affinity, but only WT GHBP bound bGH. Cross-linking of ^{125}I-labeled hGH to WT or mutant GHR produced a 141-kDa labeled complex whose appearance was blocked by unlabeled hGH, but bGH blocked cross-linking only to WT receptors. Both hGH and bGH stimulated tyrosine phosphorylation of a 95-kDa protein in cells transfected with WT GHR, but bGH was less effective in cells expressing mutant GHR. We conclude that incompatibility of Arg^{43} in the hGHR with His^{171} in nonprimate GH is the major determinant of species specificity.**

With one notable exception, peptide and protein hormones produced in one mammalian species are physiologically effective when injected into another species. Humans and monkeys respond only to growth hormone (GH) derived from primates, but primate GH is fully effective when administered to other mammalian species (1, 2). This phenomenon has been called "species specificity." Detailed knowledge of the amino acid sequences of GH from a variety of mammalian species accumulated over the past two decades has provided an understanding of the similarities and differences between the GHs of different species (3) but failed to provide insight into the molecular basis of species specificity. Explanations that have been proposed include variations on the idea that all species of GH contain a common biologically active core that must be released to be functional or that is blocked from acting on target cells by constituents on the hormone or receptor (3, 4). Recent dramatic advances in our understanding of the primary and secondary structures of GH and its receptor have made it fruitful to revisit the species specificity phenomenon. Cloning of the human (h) and rabbit GH receptors (GHRs) by Leung *et al.* (5) led to the elaboration of the amino acid sequences of the bovine (b) (ref. 6 and GenBank accession no. X70041), porcine (8), ovine (9), rat (10, 11), and mouse (12) GHRs. The complete amino acid sequences of GH molecules of at least 33 vertebrate species are also known (13). Of greater importance, perhaps, than the amino acid sequences of GH and its receptor, is their three-dimensional structure, which determines the juxtapositioning of amino acids in space rather than in linear sequence. Solution of the crystallographic structure of porcine GH (14) provided essential information about how the GH molecule folds. In addition, high-resolution mutational analysis (15, 16) of the hGHR and x-ray crystallographic studies of the GHR complex have established that GH binds to its receptor in a 1:2 ratio (17–19). These studies defined the two surfaces of the GH molecule and the two nearly identical surfaces of the receptor molecules that come into direct contact when the hormone binds.

If these findings for the human hormone and receptor are generally applicable, it is possible that the molecular basis for species specificity may reside in the 6 amino acid residues in the GHR that form hydrogen bonds and salt bridges with 8 amino acids in binding site 1 on the GH molecule (19). Comparison of these residues in the hGHR with those in receptors of different species reveals that at only one locus is there a nonconservative amino acid substitution that is unique for the human form of the receptor (Table 1). Residue 43 in the hGHR is Arg (Arg^{43}), which bears a strong positive charge at physiological pH, whereas receptors from other mammalian species contain the neutral amino acid Leu at this location. Two amino acid residues in site 1 of human GH make contact with Arg^{43} of the receptor: Asp^{171}, which is negatively charged at physiological pH, and Thr^{175}. Thr^{175} appears to be strictly conserved, but the locus corresponding to Asp^{171} in hGH and monkey GH is occupied by His in GH molecules of fish, amphibia, reptiles, birds, and all nonprimate mammals (13) (Table 2). His has a slight positive charge at pH 7.4. Of the 8 amino acids in site 1 of GH that come within ≈3 Å of amino acids in the receptor, the His → Asp in hGH is the only one that involves a significant change in properties.

These considerations suggested that incompatibility of Arg^{43} of the receptor with His^{171} of GH interferes with binding of nonprimate GH at site 1 of the hGHR. To test this idea, we mutated Leu^{43} of nonprimate receptors to Arg to determine whether this change would limit their ability to bind nonprimate GH. For this purpose we used the full-length bGHR (accession no. X70041) and the alternately spliced short isoform of the rat GHR (20) that lacks a transmembrane domain and is secreted as the GH binding protein (GHBP) (10). While these studies were in progress, we became aware of the results of two other studies of similar intent. In their studies of the importance of the first disulfide loop for GH binding, Gobius *et al.* (21) mutated various residues of the rabbit GHR between residues 39 and 47, including Leu^{43} → Arg, and found a small decrease in the binding affinities for both b- and hGH. Laird *et al.* (22), however, reported that hGHR acquired the ability

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Abbreviations: GH, growth hormone; GHR, growth hormone receptor; GHBP, growth hormone binding protein; h, human; b, bovine; BSA, bovine serum albumin; WT, wild type.

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Table 1. Amino acids in site 1 of the GHR expected to make contact with GH

Species	Residue 43	103	104	120	127	165	Ref(s).
Bovine	Leu	Val	Trp	His	Asp	Val	5, 6
Human	Arg	Ile	Trp	Glu	Glu	Ile	4
Mouse	Leu	Ile	Trp	Gln	Glu	Val	11
Porcine	Leu	Ile	Trp	Gln	Glu	Val	7
Rabbit	Leu	Ile	Trp	Gln	Glu	Val	4
Rat	Leu	Ile	Trp	Glu	Glu	Val	9

to bind bGH when Arg^{43} was mutated to Leu. We report here our findings on the behavior of rat GHR and bGHR after the $Arg^{43} \rightarrow$ Leu substitution.

MATERIALS AND METHODS

Construction of Expression Plasmids and Mutagenesis. Full-length bGHR cDNA coding sequence (accession no. X70041) was obtained by a reverse transcription–polymerase chain reaction (PCR; FastTrack mRNA isolation kit, Invitrogen) using poly(A) RNA extracted from *Bos indicus* livers as template. The nucleotide sequences of the PCR primers correspond to conserved regions in the hGHR and rabbit GHR cDNAs (5). The cDNA was subcloned into a pBR322-derived mammalian expression vector, pMet-IG7 (X.Z.W., unpublished data), that contains the mouse metallothionine transcriptional regulatory elements upstream of the bGHR insert and the bacteriophage f1 intergenic region. The resulting phagemid was termed pMet-IG-bGHR.

cDNA encoding the soluble rat GHBP (20) was subcloned into a p-Alter-1 phagemid (Promega) to obtain the plasmid p-Alter-GHBP. Single-stranded DNA from pMet-IG-bGHR and p-Alter-GHBP was isolated as described (23). Oligonucleotide-directed mutagenesis (24) of bGHR and GHBP cDNA was performed using oligonucleotides (5′-TCCCCT-GAACGGGAGACCTTT-3′) encoding the change (underlined) $Leu^{43} \rightarrow$ Arg. The resulting mutations were confirmed by DNA sequencing. Wild-type (WT) and mutant GHBP cDNAs were assembled in mammalian expression vectors that contain a cytomegalovirus promotor and a neomycin-resistance gene (pRC-CMV, Invitrogen).

Cell Culture and GHR Stable Cell Lines. Mouse L cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO/BRL) containing 10% (vol/vol) calf serum (HyClone) at 37°C and were used to establish stable lines as described (24). Mutant and WT pRC-CMV-GHBP plasmids were transfected into mouse L cells by using Lipofectin (GIBCO/BRL). Neomycin-resistant cells growing in the presence of Geneticin (GIBCO/BRL; 200 μg/ml) were cloned by an infinite dilution procedure (24). Clones with the highest expression were selected from results of RNA slot blot and ^{125}I-labeled hGH binding assays (see below). Clonal lines expressing WT bGHR (bGHR-S2), mutant bGHR-Leu^{43}Arg (bGHR-S11), WT rat GHBP (rGHBP-S7), or mutant rat GHBP-Leu^{43}Arg (rGHBP-S9) were used in the following studies.

Table 2. Amino acids in hGH that make contact with site 1 of the GHR and residues in equivalent loci in other species

Species	Residue 41	46	61	167	168	171	175	178
Bovine	Arg	Gln	Pro	Arg	Lys	His	Thr	Arg
Bullfrog	Arg	Lys	Pro	Lys	Lys	His	Thr	Lys
Chicken	Arg	Lys	Pro	Lys	Lys	His	Thr	Lys
Flounder	Arg	Lys	Pro	Lys	Lys	His	Thr	Thr
Human	Lys	Gln	Pro	Arg	Lys	Asp	Thr	Arg
Monkey	Lys	Gln	Pro	Arg	Lys	Asp	Thr	Arg
Ovine	Arg	Gln	Pro	Arg	Lys	His	Thr	Arg
Porcine	Arg	Gln	Pro	Lys	Lys	His	Thr	Arg
Rat	Arg	Gln	Pro	Lys	Lys	His	Thr	Arg
Salmon	Arg	Gln	Pro	Lys	Lys	His	Thr	Thr
Shark	Arg	Gln	Pro	Lys	Lys	His	Thr	Lys
Turtle	Arg	Lys	Pro	Lys	Lys	His	Thr	Lys

Binding Assays. bGHR-S2 and bGHR-S11 cells were propagated to between 50 and 100% confluence ($0.25–0.5 \times 10^6$ cells) in 12-well tissue culture plates. Binding assays were performed as described (25). Briefly, monolayers were depleted of GH by incubation in serum-free DMEM for 1 h at 37°C. Approximately 250,000 cpm of ^{125}I-labeled hGH (2 ng/ml) was then added to each well without or with various amounts of unlabeled hGH or bGH in a total volume of 0.5 ml of Krebs–Ringer phosphate buffer (KRP; pH 7.4) that contained 0.1% bovine serum albumin (BSA; GBC, Northlake, IL). After incubation for 1 h at room temperature, the cells were rinsed three times with KRP, solubilized in 1 ml of 1% SDS, and transferred to counting tubes for measurement of radioactivity with a multiwell γ counter.

rGHBP-S7 and rGHBP-S9 cells were plated in 75-cm^2 flasks. GHBP released into the incubation medium was quantitated as described (20). One day after the cells reached confluence, 100 μl of medium was transferred to tubes that contained 250,000 cpm of ^{125}I-labeled hGH (2 ng/ml) without or with different amounts of unlabeled hGH or bGH in Tris-buffered saline containing 0.1% Triton X-100 and antiserum 1615 (1:1000 dilution) raised against the hydrophilic C terminus of rat GHBP (20). After overnight incubation at room temperature, immune complexes were collected on protein A-agarose beads and radioactivity was measured as described above.

Cross-Linking Studies. bGHR-S2 and bGHR-S11 cells were grown to confluence in 25-cm^2 tissue culture flasks and depleted of GH as described above. Cross-linking of ^{125}I-labeled hGH to receptors was performed as described (26). Cells were incubated with 500,000 cpm of ^{125}I-labeled hGH (≈5 ng/ml) in KRP containing 0.1% BSA at room temperature for 2 h without or with unlabeled hGH (5 μg/ml) or bGH (5 μg/ml) and then washed three times with KRP. Freshly prepared water-soluble cross-linking reagent bis(sulfosuccinimidyl)suberate (BS_3; Pierce) dissolved in KRP to 1 mM was added and the cells were incubated for 30 min at 4°C. Cells were homogenized in 1 ml of 0.25 M sucrose/1 mM EDTA in the presence of a mixture of the following protease inhibitors (each at 2 mM): phenylmethylsulfonyl fluoride, "N^{α}-(*p*-tosyl)lysine chloromethyl ketone," and L-1-tosylamido-2-phenylethyl chloromethyl ketone (Sigma). The samples were then centrifuged at 15,000 × *g* for 10 min. The pellets were solubilized in SDS/PAGE sample buffer and loaded onto 7.5% gels (27). After electrophoresis, the gels were dried and exposed to x-ray film.

Western Blot Analysis. GH-dependent tyrosine phosphorylation was studied by Western blot analysis with a horseradish peroxidase-conjugated anti-phosphotyrosine serum (PY20, ICN) as described (28). bGHR-S2 and bGHR-S11 cells were grown to ≈80% confluence (0.8×10^6 cells) in 6-well tissue culture plates. Monolayers were incubated overnight in DMEM containing 0.5% calf serum. After addition of hGH or bGH, the cells were incubated at 37°C for 10 min. Cells not treated with GH served as controls. Incubations were terminated by washing the cells with phosphate-buffered saline, and cells were solubilized in 250 μl of lysis solution (50 mM Tris·HCl, pH 6.8/1% SDS/1% 2-mercaptoethanol/0.1 M dithiothreitol/5% sucrose/0.1 mM sodium orthovanadate) heated to 90–100°C. Cell lysates were passed through a 27-gauge needle 10 times to shear DNA. Cell lysates (30 μl) were mixed with 1 μl of 0.6% bromophenol blue and subjected to SDS/PAGE (26) on 7.5 gels in a mini-gel system (SE-250,

Hoefer). After electrophoresis, gels were rinsed twice with blotting buffer (25 mM Tris·HCl/192 mM glycine/20% methanol/0.075% SDS/0.5 mM sodium orthovanadate) and transferred to a Hybond-ECL membrane (Amersham) for 3 h at a constant voltage of 45 V. Hybond-ECL membrane blots were rinsed twice with rinsing buffer (RB: 10 mM Tris·HCl, pH 7.5/75 mM NaCl/0.1% Tween 20/1 mM EDTA). Blots were incubated overnight in blocking solution (RB/4% BSA; Boehringer Mannheim) and then incubated for 1 h with PY20 at 0.1 μg/ml in blocking solution. After incubation, blots were washed three times with RB and developed using enhanced chemiluminescence (ECL) reagent (Amersham).

RESULTS

Stably transfected mouse L cells expressed 70,000 ± 10,000 WT (bGHR-S2 cells) or 88,500 ± 3000 mutated (bGHR-S11 cells) bGHRs on their surfaces as determined by Scatchard analysis (29). Cells that expressed the short isoform of the rat GHR were unable to bind GH but secreted ≈24,000 copies of WT (rGHBP-S7 cells) or ≈37,000 copies of mutated (rGHBP-S9 cells) GHBP into the culture medium per cell per day, as judged by Scatchard analysis. Mouse L cells transfected with only the vector neither bound ^{125}I-labeled hGH nor secreted GHBP into the medium. The K_d for hGH was 2.2 ± 0.53 and 2.4 ± 0.6 nM for the WT and mutant bGHRs, respectively, and 0.88 ± 0.42 and 0.30 ± 0.16 nM for the WT and mutant GHBP.

Binding Assays. Conditioned medium in which rGHBP-S7 or rGHBP-S9 cells had grown for 2 days contained sufficient GHBP to bind ≈10% of the ^{125}I-labeled hGH trace, and both the WT and the mutated receptors bound hGH with nearly equal affinity (Fig. 1). Unlabeled hGH and bGH were equally effective in competing with the trace for sites on the WT GHBP and reduced binding by 50% when present at concentrations of ≈3 nM (Fig. 1*A*). In contrast, bGH produced only ≈30% displacement of ^{125}I-labeled hGH from the mutated GHBP even at bGH concentrations as high as 5 μM (10 μg/ml) (Fig. 1*B*).

A similar, but less dramatic, effect of mutating Leu^{43} to Arg was seen in the binding of ^{125}I-labeled hGH to mouse L cells expressing WT or mutated bGHRs. Both bGHR-S2 and bGHR-S11 cells bound ≈10% of the ^{125}I-labeled hGH trace in the absence of unlabeled GH, and hGH and bGH were equally effective in competing for binding sites on bGHR-S2 cells (Fig. 2*A*). In contrast, while hGH displaced 50% of the bound ^{125}I-labeled hGH from bGHR-S11 cells at 1 nM, a >200-fold higher concentration of bGH (≈0.2 μM) was needed to achieve the same degree of displacement from these cells (Fig. 2*B*).

Cross-Linking Studies. When bGHR-S2 or bGHR-S11 cells were incubated with ^{125}I-labeled hGH at 5 ng/ml and then treated with the bifunctional cross-linking reagent bis(sulfosuccinimidyl)suberate, a complex of ≈141 kDa was formed (Fig. 3, lanes D and G). No such complex was apparent in parental mouse L cells (Fig. 3, lane A). Addition of unlabeled hGH or bGH (5 μg/ml) along with the labeled hormone blocked the appearance of the 141-kDa band in extracts of bGHR-S2 cells, indicating that a 1000-fold excess of either hormone displaced the labeled hormone from its binding sites (Fig. 3, lanes E and F). In contrast, only hGH blocked the appearance of the labeled band in bGHR-S11 cells (Fig. 3, lanes H and I), but, in agreement with results shown in Fig. 2*B*, the intensity of the band was reduced by this high concentration of bGH.

Signal Transduction. Earlier studies revealed that transfection of mouse L cells with cDNA for the GHR resulted in the acquisition of the capacity to phosphorylate a 95-kDa protein (pp95) on tyrosine residues in response to stimulation with GH (28). To determine whether the data on hormone binding is relevant to expression of a GH signal in these cells, we treated bGHR-S2 and bGHR-S11 cells with h- and bGH and tested for tyrosine phosphorylation of pp95. Cells were grown to confluence in 6-well plates and serum-depleted overnight before treatment for 10 min with hGH or bGH at 100 or 500 ng/ml (Fig. 4). Tyrosine phosphorylation of pp95 was evaluated by Western blot analysis after dissolving the cells in SDS and separating the proteins by PAGE. In bGHR-S2 cells, both hGH and bGH increased the phosphorylation of pp95 in a concentration-dependent manner, although bGH appeared to elicit a somewhat stronger response than hGH in seven experiments. hGH elicited similar responses in bGHR-S11 cells, while bGH evoked a much weaker stimulation of pp95 phosphorylation than hGH.

DISCUSSION

Because they express few, if any, GHRs (24, 28), mouse L cells, the parental cells of rGHBP-S7, rGHBP-S9, bGHR-S2, and

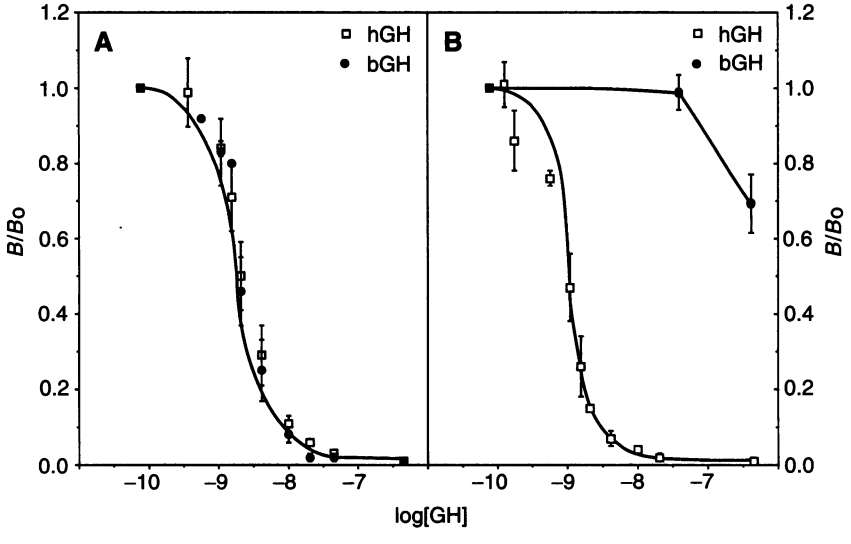

FIG. 1. Competition for binding to GHBP secreted by rGHBP-S7 (*A*) or rGHBP-S9 cells (*B*). Aliquots of culture medium were incubated overnight with ^{125}I-labeled hGH (≈250,000 cpm; 2 ng/ml), the indicated concentrations of unlabeled hGH or bGH, and antiserum 1615. Immune complexes were collected with protein A-agarose beads and radioactivity was measured. Each point represents the mean ± SEM for two to five experiments (each in triplicate). *B*, bound; B_o, bound in absence of competitor.

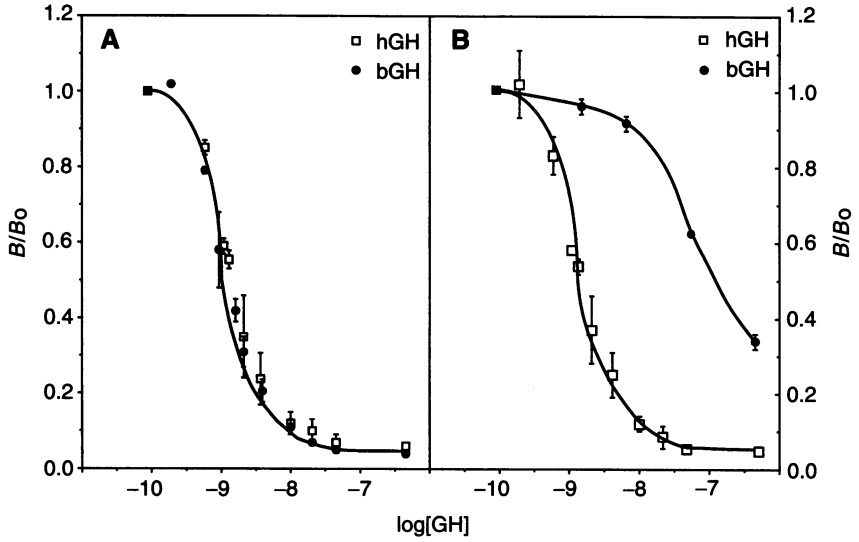

FIG. 2. Competition for binding to bGHR-S2 cells (*A*) and bGHR-S11 cells (*B*). Confluent cells in 12-well plates were incubated for 1 h at room temperature with ^{125}I-labeled hGH (≈250,000 cpm; 2 ng/ml) and the indicated concentrations of unlabeled hGH or bGH. The cells were rinsed three times in buffer and dissolved in SDS, and radioactivity was measured. Each point represents the mean ± SEM obtained in four experiments (each in triplicate).

bGHR-S11, are convenient for expressing transfected GHRs and evaluating the binding characteristics of both the transmembrane and secreted isoforms. The transfected receptors appear to be fully glycosylated and, hence, are preferable to the bacterial expression system for these studies since carbohydrate moieties may potentially contribute to species-specific binding. We have shown that the WT rat GHBP is secreted by rGHBP-S3 cells and binds hGH and bGH with the same high affinity. In contrast, the mutant GHBP secreted by rGHBP-S9 cells binds hGH and WT GHBP but has little affinity for bGH. Transfection of these cells with the bGHR resulted in abundant expression of both WT and mutant receptors. Furthermore, when transfected with cDNA encoding these receptors, mouse L cells gain the capacity to respond to GH. Although the physiological role of pp95 and the consequences of its phosphorylation on tyrosine residues are not yet known, the reaction is nevertheless a useful marker of GH signal transduction.

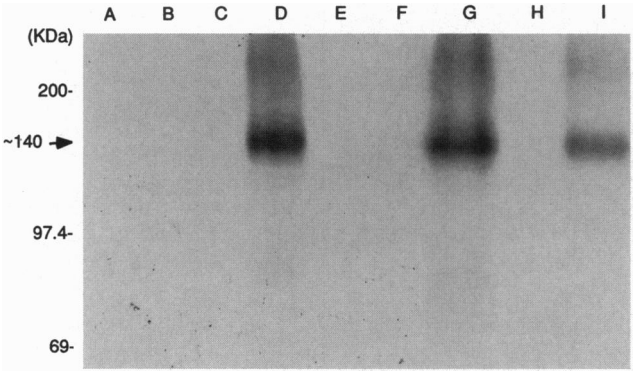

FIG. 3. Cross-linking of bGHR-S2 and bGHR-S11 cells. Confluent cells in 25-mm tissue culture flasks were incubated with ^{125}I-labeled hGH (5 ng/ml) in the absence or presence of unlabeled hGH or bGH. After 1 h, the cells were cross-linked with 1 mM bis(sulfosuccinimidyl)suberate, solubilized, and subjected to SDS/PAGE on 7.5% gels. Lanes: A, mouse L cells; B, mouse L cells/hGH; C, mouse L cells/bGH; D, bGHR-S2 cells; E, bGHR-S2 cells/hGH; F, bGHR-S2 cells/bGH; G, bGHR-S11 cells; H, bGHR-S11 cells/hGH; I, bGHR-S11 cells/bGH.

The present results support the proposition that Arg^{43} found thus far only in the hGHR is the major determinant of the phenomenon of species specificity. Mutation of Leu to Arg in the corresponding position of two nonprimate GHRs dramatically decreased their ability to bind bGH without affecting their affinity for hGH. For the bovine receptor, this single change increased the EC_{50} for displacement of ^{125}I-labeled hGH by ≈200-fold. The effect on the rat GHBP was at least 10-fold greater. By way of comparison, bGH was ≈3000 times less potent than hGH in competitive binding studies using the human IM-9 lymphocyte cell line (30), suggesting that the combined effects of all of the other differences in amino acid sequence account for another 15-fold change in affinity. These results are also in harmony with the report of Laird *et al.* (22) who performed the complementary experiment and found that mutating Arg^{43} to Leu in the hGHR enabled it to bind bGH. The finding that mutation of the Arg^{43} to Ala in the human receptor caused a <2-fold reduction in its affinity for hGH (16) is not in conflict with the present findings since hGH binds perfectly well to nonprimate receptors that have another neutral amino acid, Leu, in this locus. Thus Arg^{43} appears to act more as a hindrance to the binding of bGH than as a facilitator of the hGH binding. This is in accord with the suggestion of Barnard *et al.* (31) on theoretical grounds that an additional positive charge on the primate GHR might prevent binding of nonprimate GH to the primate receptor. Unfortunately, no effects on bGH binding were reported for the receptors with the Arg → Ala mutation (16).

The finding of Gobius *et al.* (21) that mutating Leu^{43} to Arg caused only a small and parallel decrease in the affinity of the rabbit GHR for hGH and bGH is somewhat puzzling. Except for position 43, the amino acid sequences in the human, bovine, rabbit, and rat GHRs are identical in the stretch between residues 35 and 49, which includes the first disulfide loop in its entirety (14). Furthermore, five of the six amino acid residues that are thought to make contact with GH in site 1 and their nearest neighbors are identical in rabbit and rat receptors (refs. 6, 10, and 11 and Table 1). The negatively charged Glu^{120} in the third disulfide loop of the hGHR and its equivalent in rat receptors is replaced by the uncharged Gln of the rabbit receptor (Table 1), but whether this or other differences can compensate in some way for the Leu → Arg mutation is not known. From the studies of Lowman and Wells (32) in which

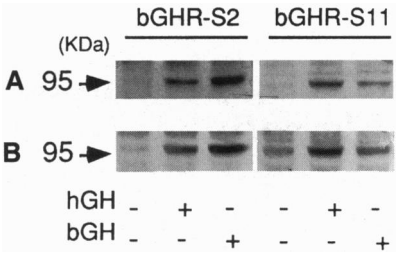

FIG. 4. Western blot analyses of GH-dependent tyrosine phosphorylation of protein (pp95) in bGHR-S2 and bGHR-S11 cells. Cells in 6-well plates were incubated for 10 min with hGH or bGH at 100 (*A*) or 500 (*B*) ng/ml. Cellular proteins were separated by SDS/PAGE, transferred to nitrocellulose, and reacted with antiserum PY20. Similar data were obtained in four experiments of similar design.

selective point mutations in GH increased receptor binding, it is clear that even small changes in amino acid side chains can significantly alter binding. These considerations underscore the likelihood that other loci in the receptor in addition to Arg^{43} may also contribute to species specificity. On the other hand, it is noteworthy that the ovine placental lactogen binds to hGHRs in liver membranes and IM-9 cells with nearly the same affinity as hGH (33, 34) although the two hormones have only ≈26% identity in their amino acid sequences and differ in five of the eight amino acids that make close contact with the receptor at site 1 (34, 35). Ovine placental lactogen is thought to be similar to GH in its three-dimensional configuration but differs from all of the GHs in having a Ser in the locus equivalent to 171 (34) instead of the Asp of hGH and the His of all of the nonprimate GHs.

The data presented here lend strong support to the idea that incompatibility of Arg^{43} in the receptor with His^{171} in the hormone is the major, albeit not the only, factor that accounts for the low affinity of the hGHR for nonprimate GH molecules. Minimal changes in the DNA encoding the hormone and the receptor thus produced a profound change in biological activity. Although there are multiple codons for each, the codons for Leu and Arg and for His and Asp each can differ by a single base. In evolutionary terms, the His → Asp mutation in the hormone must have preceded the Leu → Arg mutation in the receptor. It is likely that the ancestral form of GH contained His rather than Asp, since His is found in the equivalent position relative to the disulfide forming the large loop in prolactin and in GH of vertebrate species ranging from cartilagenous fish to mammals (36). The His → Asp mutation, however, probably occurred early in primate evolution since all five members of the primate GH gene family (7, 37) code for Asp at this locus. The subsequent Leu → Arg mutation in the receptor might have provided the selection pressure to maintain the Asp^{171}, but the factors that selected for this change in the receptor are not at all apparent.

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