



Effect of fasting and growth hormone(GH)administration on GH receptor(GHR)messenger ribonucleic acid(mRNA)and GH-binding protein(GHBP)mRNA...

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**EFFECT OF FASTING AND GROWTH HORMONE (GH)
ADMINISTRATION ON GH RECEPTOR (GHR) MESSENGER
RIBONUCLEIC ACID (mRNA) AND GH-BINDING PROTEIN (GHBP)
mRNA LEVELS IN MALE RATS**

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(雄ラット成長ホルモン (GH) 受容体およびGH結合蛋白mRNA

に対する絶食とGH投与の効果)



Pergamon



EFFECT OF FASTING AND GROWTH HORMONE (GH) ADMINISTRATION ON GH RECEPTOR (GHR) MESSENGER RIBONUCLEIC ACID (mRNA) AND GH-BINDING PROTEIN (GHBP) mRNA LEVELS IN MALE RATS

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Summary

To elucidate whether GHR and GHBP are coordinately regulated or not, we studied the effect of fasting with or without GH administration on the GHR and GHBP mRNAs in the liver as well as in extrahepatic tissues in rats. Tissues were collected from 7-week-old male rats by decapitation 1, 3, and 7 days after the start of fasting. Liver GHR mRNA levels were not affected 1 day after the start of fasting but progressively decreased for the subsequent 3 and 7 days of fasting as compared with those in control rats fed ad libitum. In contrast, liver GHBP mRNA levels significantly rose after 1 day fasting, returned to the control level after 3 days and further reduced after 7 days of fasting. Changes in GHBP mRNA level after fasting were different among the tissues. A transient increase in GHBP mRNA levels was observed in muscle and heart as well as liver, while the GHBP mRNA levels in fat tissues did not change throughout 7 days of fasting. Next, bovine GH(bGH) was administered ip to the fasted rats and control fed rats for either 1 day(100mg, tid) or 5 days(150mg, daily). In fed rats, liver GHR mRNA level was significantly increased by 1 day bGH treatment, but after 5 days treatment with bGH it was not different from the level in saline-injected control. Accordingly, net increment of plasma IGF-I was 296.0 ng/ml with 1 day bGH treatment and 234.2 ng/ml with bGH administration for 5 days. In fasted rats, liver GHR mRNA level did not changed after 1 day treatment with bGH, but markedly decreased 5 days after bGH administration. Net increment of plasma IGF-I was slightly reduced to 284 ng/ml with 1 day treatment with bGH, and markedly decreased to 37.0 with bGH administration for 5 days. The effect of GH administration on liver GHBP mRNA level was virtually absent in either fasting or fed state. These findings suggest that GHR and GHBP mRNAs in the liver are expressed in different ways and that expression of GHBP mRNA is differently regulated among tissues.

Key Words: fasting, growth hormone, mRNA, GH-binding protein

GHBP is an alternatively spliced product of GHR gene in rat and mouse(1, 2), but it appears to arise from proteolytic cleavage of GHR in human and rabbit(3). Furthermore, since biological activity of GH is influenced by the level of GHR as well as GHBP, it is of importance to elucidate whether GHR and GHBP are coordinately regulated or not.

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In rats, GHBP mRNA is found in the liver as well as in all extrahepatic tissues that contained GHR mRNA. However, the ratio of GHR and GHBP mRNAs varied among tissues(4, 5). During pregnancy GHBP mRNA increases whereas GHRmRNA do not change in rat and mouse liver, suggesting distinct regulation of GHR and GHBP mRNAs(5, 6). The separate regulation of GHR and GHBP mRNAs was also reported on developmental profiles in rat kidney and lung(7).

It is generally known that plasma GHBP and IGF-I levels as well as hepatic GHR levels are decreased in fasting state. However, the precise mechanism by which plasma IGF-I level is lower in fasting remains to be clarified. This may result from attenuation of GH action, e.g. through decreased GH secretion, down-regulation of hepatic GHR and postreceptor impairment in signal transduction, changes of GHBP levels etc. or from a mechanism independent of GH action like a direct effect of nutritional factors on IGF-I synthesis.

In the present study we prepared two different cDNA probes corresponding to the intracellular domain of GHR mRNA and to the hydrophilic carboxy-terminus of GHBP mRNA, respectively, using a reverse transcription-polymerase chain reaction(RT-PCR) method which had been also used to detect low level of the mRNAs. The effect of fasting and GH administration on GHR and GHBP mRNA levels were examined in the liver as well as in extrahepatic tissues in rats by Northern blot analysis to elucidate whether the expression of GHR and GHBP mRNAs in the liver are coordinately regulated by fasting and GH administration or whether the GHBP mRNA levels are regulated in a similar manner among different tissues.

Methods

Animals. Male Sprague-Dawley rats(Keari Co. Ltd., Osaka, Japan), aged 6 weeks were housed under controlled temperature(22 C) and light conditions(on at 0600, off at 1800) with standard chow(Oriental yeast Co.Ltd., Tokyo, Japan) and water ad libitum for several days before experiments.

Experimental design. Experiment 1 Rats aged 7 weeks were divided into fasting(n=4) and control (n=4) groups. They were allowed free access to water throughout the study. Rats were killed by decapitation before and 1, 3, and 7 days after the start of fasting. Liver was collected upon sacrifice. Heart, muscle, and perirenal fat tissues were also obtained at each sacrifice except on the 7th day. The tissues were immediately frozen in liquid nitrogen and kept at -70 C until RNA extraction. The contents of liver GHR mRNA and of GHBP mRNA in various tissues were quantitated by Northern blot analysis. Trunk blood was collected in heparinized plastic tubes after decapitation and centrifuged (3000 rpm for 10 min at 4 C). The plasma was stored at -20 C until assayed for IGF-I and rGH. Daily changes in body weight were monitored.

Experiment 2 Rats aged 7 weeks were divided into fasting and control groups. Each group of rats was further subdivided into two groups; one group was injected with bovine GH [bGH, USDA-bGH-B-1 (AFP5200) 1.4 IU/mg, and another received saline alone. Rats were subjected to fasting for 1 day (experiment 2-a) or 5 days (experiment 2-b). In experiment 2-a, fasting started at 1600 h and either 100 mg bGH or saline (0.1ml) was injected ip at the same time(1600 h), followed by two additional injections every 8 h (0000, and 0800 h). Rats of four groups were decapitated at 1600h(1 day after the start of fasting). In experiment 2-b, fasting started at 2000h and either 150 mg bGH or saline (0.15 ml) was administered ip every morning (0800h) for 5 successive days. Rats were sacrificed by decapitation at 2000h on the fifth day. In both experiments liver was dissected for determination of GHR and GHBP mRNA levels, and trunk blood was collected for RIA of IGF-I. Body weight of the rat was recorded everyday.

Preparation of probes. Two pairs of primers, GHR1/GHR2 and GHBP1/GHBP2 (FIG.1), were synthesized by the methoxyphosphoamidite method on an Applied Biosystems 381A DNA synthesizer and were used to prepare the probes specific for GHR and GHBP mRNA by a RT-PCR method as described previously(8). Sequence of four primers was as follows: GHR1(a sense oligonucleotide), 5'-AGCAGTCCCGTGTCACTGGCAAAC, 24-mer with 5' end at 1426 on rat GHR cDNA nucleotide sequence (1); GHR2(an antisense oligonucleotide), 5'[CTCGAATTC]TCTGTGGTGATGTAAATGTCTCT, [Eco RI linker]24-mer with 5' end at

1709 flank a specific region of GHR (intracellular domain); GHBP1 (a sense oligonucleotide), 5'-[TGTAACACGACGGCCAGT] ACCCAAGTTCAATT CCCAGCAC CC, [M13-20 primer sequence]24-mer with 5' end at 789 on rat GHBP cDNA nucleotide sequence(1); GHBP2 (an antisense oligonucleotide); 5'-[CTCGAATTC]TTTAATTATGTGTGTATGCATGTATG,[EcoR-I linker]24-mer with 5' end at 920 flank a specific region of GHBP (C-terminus hydrophilic tail). Specific first-strand cDNA copies of GHR and GHBP mRNA in the regions were synthesized at 37°C by 30 min incubation of a 20 µl reaction mixture containing 20 µg total cellular RNA from rat liver, 50 pmoles antisense oligonucleotides (GHR2, GHBP2), 10 U RNasin (Boehringer Mannheim, Penzberg, WG), and 5 U AMV reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD). The entire 20 µl reaction mixture of reverse transcribed cDNA was combined in a 80 µl final reaction mixture for PCR amplification containing 25 pmol for each oligonucleotide primer (GHR1/ GHR2 and GHBP1/GHBP2) and 1.5 U recombinant Taq DNA polymerase (AmpliTaQ™, Perkin-Elmer Cetus). After 3 min denaturation at 95°C, 30 cycles of PCR amplification which consisted of 30 sec denaturation at 95°C, 30 sec annealing at 60°C, and 120 sec at 72°C for enzymatic extension were performed, with subsequent enzymatic extension at 72°C for 7 min using a DNA thermal cycler (Astec Co. Tokyo, Japan). After DNA amplification, the PCR mixtures were electrophoresed in 3% agarose gels (2% NuSieve, 1% SeaPlaque (FMC BioProducts, Rockland, ME)), and the amplified GHR and GHBP cDNA fragments were visualized as a single band, with a size of 293 bp and 159 bp, respectively, after ethidium bromide staining. These bands were cut and dissolved in TE buffer, then recovered as recommended previously (9). Purified GHR and GHBP cDNA fragments were blunt-ended and digested with EcoRI, then subcloned into SmaI-EcoRI digested pBluescript II SK+ (Stratagene, La Jolla, CA). The single-stranded Phagemid DNA

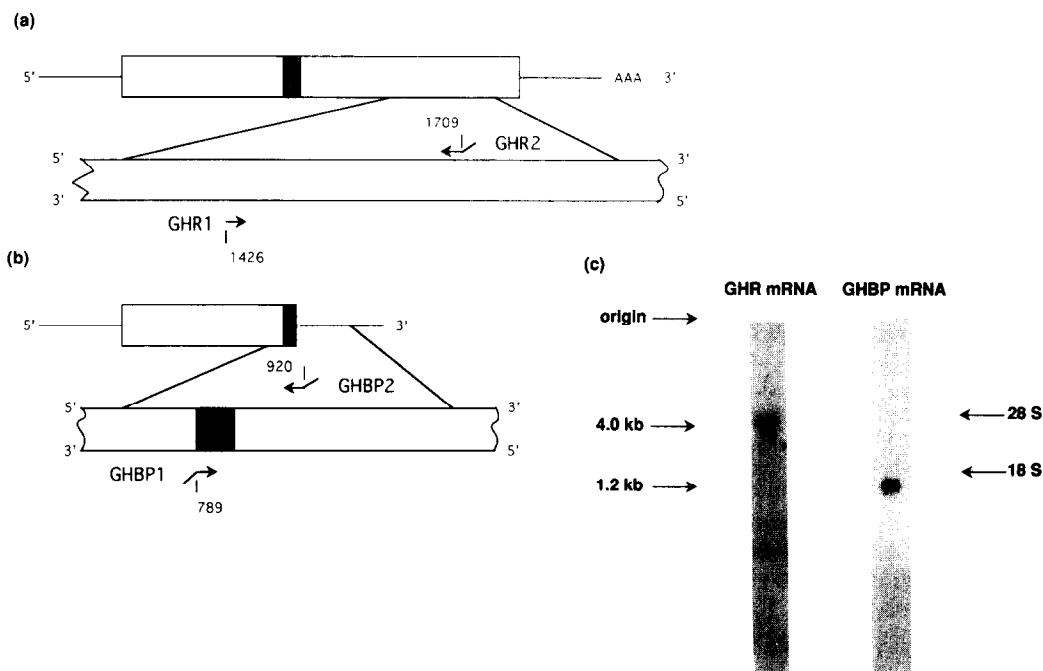


FIG.1

Schematic representation of (a) the rat GHR cDNA sequence and (b) the rat GHBP cDNA sequence. (a) The transmembrane domain is presented as a shaded area. (b) The hydrophilic tail is presented as a black box. Oligonucleotide primers GHR1/GHR2 and GHBP1/GHBP2 (horizontal arrows) are shown in the expanded portion. Diagonal tail of the arrow represents EcoRI linker in GHR2 and GHBP2, M13-primer in GHBP1. (c) A full Northern blot of rat hepatic GHR mRNA and GHBP mRNA.

containing GHR cDNA fragment was rescued by helper phage (VCS-M13) and sequenced on an Applied Biosystems 373A DNA sequencer by the dideoxy-chain termination method (8). The amplified DNA containing GHBP cDNA fragment was directly sequenced with M13-20 primer by the same way. The products of amplification were verified to correspond to the parts of the rat GHR and GHBP cDNA nucleotide sequences (1) as expected. Subcloned cDNA fragments were used as cDNA probes for GHR and GHBP after digestion with EcoRI and BamHI, respectively. The EcoRI fragment of pILaT1 was used for hybridization of α -tubulin mRNA (10). GHR, GHBP, and α -tubulin cDNA probes were labeled with α -[32 P] dCTP with a modification of the random priming method (11). Specific activity of probes ranged from 0.5-1.0 $\times 10^9$ dpm/mg DNA.

Northern blot analysis. Rat tissues were homogenized by glass homogenizers or a Polytron type 10-35 (Kinematica GmbH, Luzern, Switzerland) for approximately 10 sec. Northern blot analysis was performed as described previously (12). Total RNAs of the tissues were isolated with a slight modification of the single step acid guanidinium thiocyanate-phenol-chloroform method (13). RNA samples were diluted and denatured in the buffer composed of 50% formamide, 6% formaldehyde and 1 x MOPS [3-(N-Morpholino) propane-sulfonic acid, Dojin, Kumamoto, Japan] by heating at 55 C for 15 min. Twenty μ g RNA per lane were applied to electrophoresis through a 0.66M formaldehyde agarose (1%) gel and blotted to a Gene Screen Plus membrane (New England Nuclear, Boston, MA). The membrane filters were then baked for 3 h at 80 C in vacuo. Prehybridization was performed in a solution containing 50% formamide, 5xDenhardt, 1% sodium dodecyl sulfate (SDS) and 10 mg sheared herring sperm DNA for 2 h at 42 C. Hybridization was carried out for 16 h in the same buffer containing the probe at the concentration of 10^8 dpm/ml. Then, the filters were washed twice with 2 x SSC (1 x SSC=0.15M NaCl and 0.015 M Na citrate) containing 1% SDS for 30 min at 60 C, and followed by washing twice with 0.1 x SSC for 30 min at 20 C. The filters were exposed to Kodak X-Omat AR film (Eastman Kodak, Rochester, NY) at -80 C using intensifying screen (Lightning Plus, Dupont, Wilmington, DE). Filters were then washed twice with 0.1 x SSC + 1% SDS for 15 min at 90 C to remove the probes. After confirming no residual radioactivities were left on the filters by autoradiography, subsequent hybridization with α -tubulin cDNA probe was performed as described above. The bands of autoradiograms were quantified by an automated image processing system (Imaging Research Inc., Ontario, Canada). Normalization of each mRNA abundance to the total amount of transferred mRNA on the filter was achieved by division with the quantity of the α -tubulin mRNA. GHR and GHBP mRNA were detected as a single band of 4.0kb and 1.2kb, respectively. (FIG.1(c))

RIA. Plasma GH was measured in duplicate by double-antibody RIA using rat GH-1-5 for iodination and rat GH-RP1 as standard, as described previously (14). Plasma IGF-I concentration was measured by a specific RIA after extraction with acidified ethanol (15).

Statistical analysis. Unpaired Student's t test was used for statistical analysis. Statistical differences were considered significant at *P* values less than 0.05. ANOVA followed by F test was used in Table 1.

Results

Effect of fasting on body weight, plasma GH level, plasma IGF-1 level and hepatic GHR and GHBP mRNA levels in male rats. As shown in FIG.2, body weight was gradually but significantly decreased in fasting rats compared with the control (a). Plasma GH levels were decreased after 1 day fasting and kept low until 7th day (b). Plasma IGF-1 levels were decreased by 51% after 1 day fasting and by 74% after 3 days (c). Hepatic GHR mRNA levels were not significantly changed after 1 or 3 days fasting, but significantly decreased after 7 days. Anomalously, 1 day fasting caused a transient but significant increase in hepatic GHBP mRNA level, which was followed by a gradual decrease on 3rd and 7th days (FIG.3).

Effect of fasting on extrahepatic GHBP mRNA levels. Effect of fasting on GHBP mRNA level was also examined in extrahepatic tissues. Changes in GHBP mRNA level were different among the tissues. Fasting for 1 day caused a significant increase in GHBP mRNA level in muscle and heart but not in fat tissue (FIG.4). In heart, a significant increase in GHBP mRNA level was also observed after 3 days fasting.

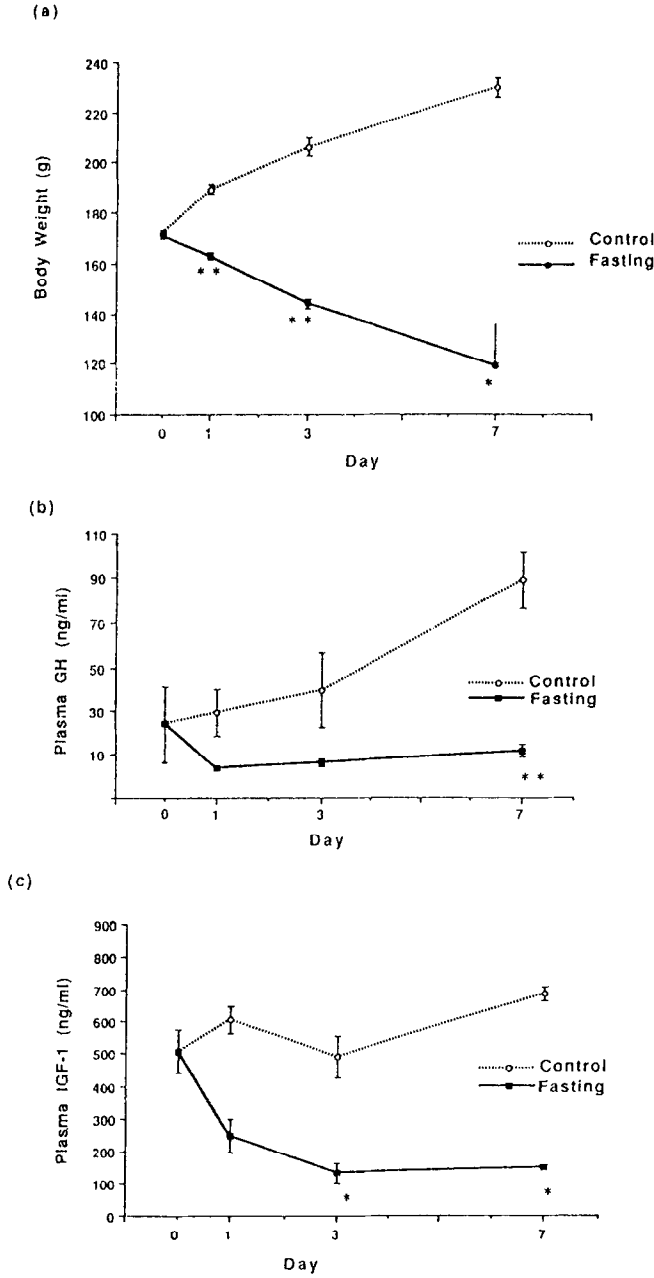


FIG.2

Change of (a)body weight, (b)plasma GH, and (c)IGF-1 levels in rats fasted or fed ad. lib.. Values are expressed as the mean \pm SEM of 4 rats in each group (* $P < 0.05$, ** $P < 0.01$ vs each day control).

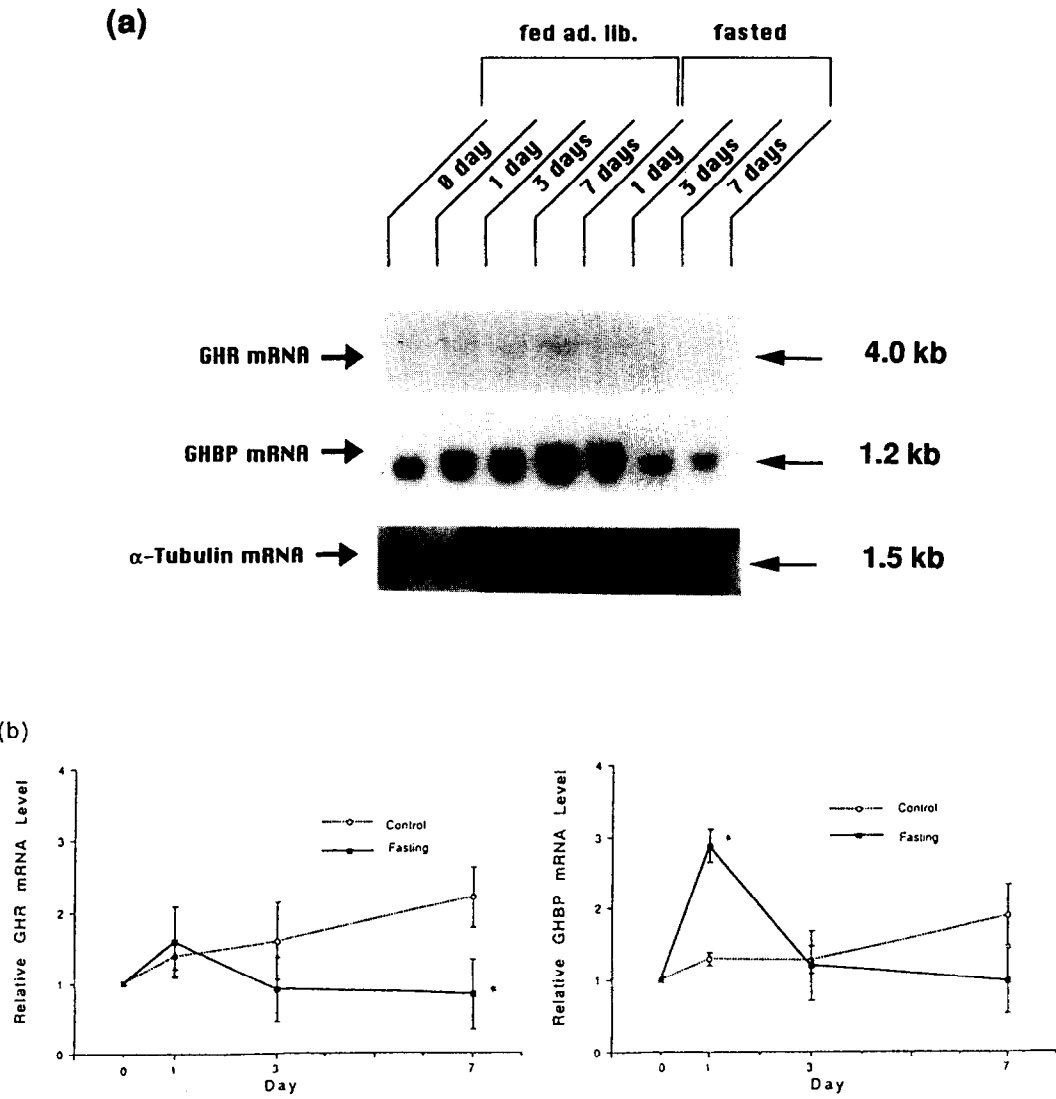


FIG.3

Northern blot analysis of hepatic GHR mRNA and GHBP mRNA in rats fasted and fed ad. lib.. (a) GHR mRNA was detected as a major band with a size of approximately 4.0 k base pairs (bp), and GHBP mRNA as a 1.2 kbp band. Each GHR and GHBP mRNA level was corrected by corresponding α-tubulin mRNA level, respectively. (b) The fold changes to the mean value of the corrected GHR and GHBP mRNA in 0 day control group was expressed as the mean ± SEM of 4 rats in each group (*P<0.05 vs each day control).

(a)

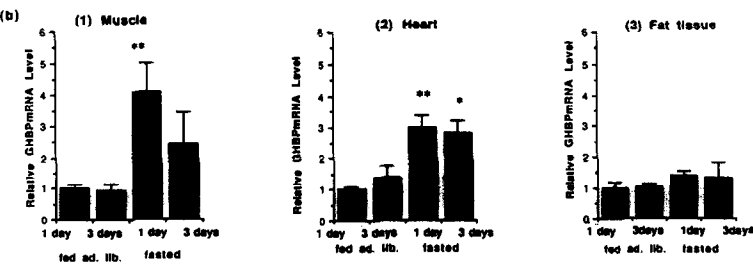
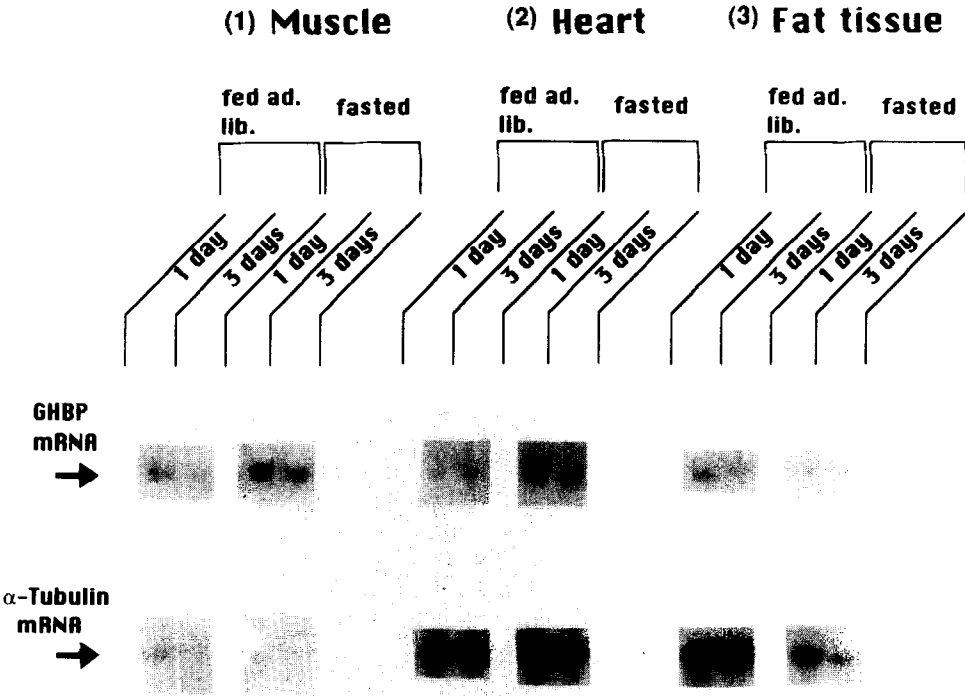


FIG.4

Northern blot analysis of extrahepatic [(1) muscle, (2)heart, (3)fat tissue] GHBP mRNA in rats fasted and fed ad. lib.. (a) GHBP mRNA was detected as a 1.2 kbp band. Each GHBP mRNA level was corrected by corresponding a-tubulin mRNA level. (b) The fold changes to the mean value of the corrected GHBP mRNA in 1 day control group was expressed as the mean \pm SEM of 4 rats in each group (*P < 0.05, **P < 0.01 vs each day control).

TABLE I

Body weight and plasma IGF-1 levels in rats fasted or fed ad. lib. for 1 day(a) or 5 days(b) with bGH or saline administration

a.	Body Weight (g)	IGF-1 (ng/ml)	b.	Body Weight (g)	IGF-1 (ng/ml)
Fasted + Saline	170.0 ± 0.4 ^{b**}	279.0 ± 42.4 ^{b*}	Fasted + Saline	112.4 ± 1.1 ^{b**}	88.6 ± 29.4 ^{b**}
Fasted + GH	176.0 ± 0.8 ^{a**} ^{b**}	563.0 ± 48.5 ^{a**} ^{b*}	Fasted + GH	110 ± 4.7 ^{b**}	125.6 ± 78.6 ^{b**}
Fed ad. lib. + Saline	195.0 ± 1.7	769.0 ± 116.5	Fed ad. lib. + Saline	212.0 ± 2.2	636.8 ± 68.9
Fed ad. lib. + GH	195.5 ± 1.3	1065.0 ± 127.7	Fed ad. lib. + GH	216.0 ± 1.4	871.0 ± 32.2 [*]

Values are expressed as mean ± SE

* $P < 0.05$

** $P < 0.01$

a vs. corresponding saline administration group.

b vs. corresponding fed ad. lib. group

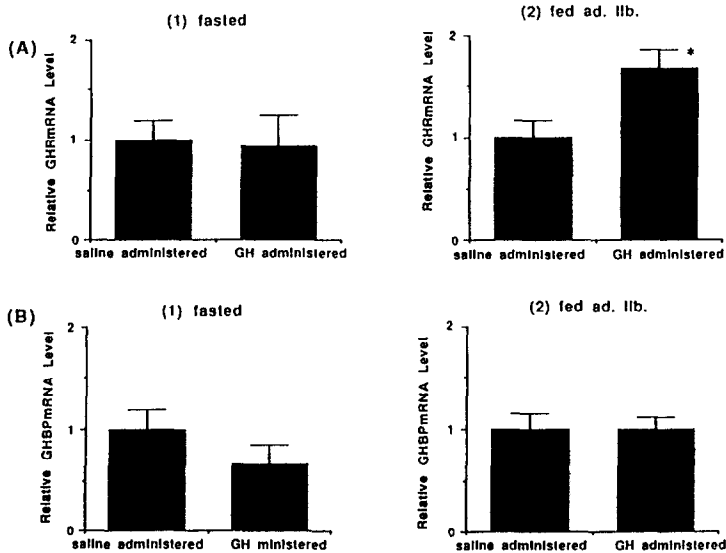


FIG. 5

Northern blot analysis of hepatic GHR mRNA and GHBP mRNA in rats fasted or fed ad. lib. with GH or saline administration for 1 day. [(1)fasted, (2)fed ad. lib. (A)GHRmRNA, (B)GHBPmRNA.]

The fold changes of GH administered group to the mean value of the corrected (A)GHR mRNA (B)GHBP mRNA in saline administered group was expressed as the mean ± SEM of rats in each group [(A)(1)saline, n=3, GH, n=4. (2)n=4. (B)(1)saline, n=3, GH, n=4. (2)n=4.].

(* $P < 0.05$, ** $P < 0.01$ vs corresponding saline administered group)

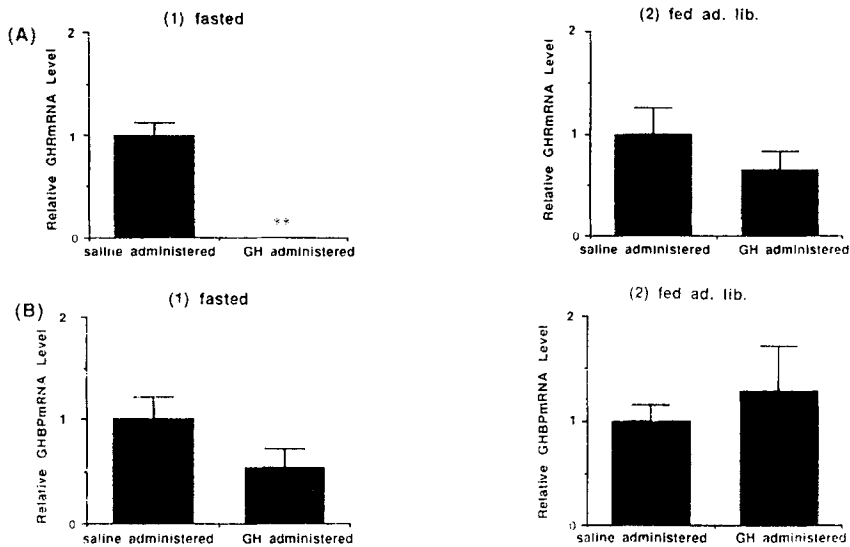


FIG.6

Northern blot analysis of hepatic GHR mRNA and GHBP mRNA in rats fasted or fed ad. lib. with GH or saline administration for 5 days. [(1)fasted, (2)fed ad. lib.,(A)GHRmRNA,(B)GHBPmRNA.]

The fold changes of GH administered group to the mean value of the corrected (A)GHR mRNA (B)GHBP mRNA in saline administered group was expressed as the mean \pm SEM of rats in each group [(A)(1)saline,n=5, GH,n=3. (2)saline,n=3, GH,n=4. (B)(1)saline,n=5, GH,n=3. (2)n=4.]. (* P < 0.05, ** P < 0.01 vs corresponding saline administered group)

Effect of GH administration on body weight, plasma IGF-I level and hepatic GHR and GHBP mRNA levels. As shown in TABLE 1., fasting for 1 and 5 days caused a significant decrease in body weight from 195 ± 1.7 to 170 ± 0 g and from 212 ± 2.2 to 112.4 ± 1.1 g, respectively, while GH administration did not affect the change in body weight in all rats except those fasting for 1 day. Plasma IGF-I levels dramatically decreased from 769 ± 116.5 to 279 ± 42.4 ng/ml and from 636.8 ± 68.9 to 88.6 ± 29.4 ng/ml by fasting for 1 and 5 days, respectively. GH administration of 100 mgx3 for 1 day caused a net increase of 296 ng/ml in plasma IGF-I level in fed rats (TABLE 1), which is consistent with up-regulated GHR mRNA level (FIG.5A). The same treatment with GH for 1 day was still effective to increase plasma IGF-I level and body weight gain in fasted rats, and the net increment, 284 ng/ml, was similar as compared with that in fed rats. GH administration of 150 mg/day for 5 days resulted in a modest increase of plasma IGF-I level in fed rats. The net increment in plasma IGF-I level, 234.2 ng/ml, however, was smaller than that after 1 day GH treatment. Up-regulation of liver GHR mRNA level, occurred after 1 day GH administration, was no longer observed in fed rats after 5 day treatment with GH. Rather, GH treatment for 5 days caused a slight decrease in GHR mRNA level in fed rats (FIG.6A). In fasted rats, daily GH administration for 5 days dramatically down-regulated GHR mRNA levels in liver (FIG.6A). The net increase of plasma IGF-I was only 37 ng/ml after 5 days GH treatment in fasted rats (TABLE 1). GH administration for either 1 day or 5 days failed to change GHBP mRNA levels in fed rats (FIG.5B, 6B), and tended to decrease GHBP mRNA level in fasted rats although the difference was not statistically significant.

Discussion

The present study demonstrated that hepatic GHR mRNA and GHBP mRNA are separately regulated by fasting and GH administration in rats. In the experiment 1, we have examined the time-

dependent effect of fasting on GHR and GHBP mRNA levels in the liver and GHBP mRNA in the extrahepatic tissues in male rats. Fasting for 1 day caused an increase in GHBP mRNA levels without significant change of GHR mRNA levels, while fasting for 7 days caused a significant decrease in GHR mRNA with a gradual decrease in GHBP mRNA. These results confirmed previous report showing a decrease in GHR mRNA by fasting in rats(16, 17). However, the regulation of GHBP mRNA was unclear in their report. The reason why fasting causes transient increase in GHBP mRNA but not GHR mRNA is unknown. We cannot completely exclude the possibility that both mRNAs were coordinately increased but that GHR mRNA is more rapidly degraded than GHBP mRNA. This assumption is supported by the previous report of Vikman et al. showing that GHR mRNA is rapidly regulated likely due to the presence of destabilizing sequences(ATTT) in the 3'-untranslated region of the rat GHR cDNA(18).

Concerning the mechanism by which 1 day of fasting causes an increase in GHBP mRNA, we examined the effect of GH replacement on GHBP mRNA in fasted rats, since plasma GH level was decreased in fasted rats. However, GH administration failed to change GHBP mRNA level. Thus, an increase in GHBP mRNA during an early period of fasting is probably independent upon the decrease in plasma GH level.

Plasma IGF-I level begins to decrease immediately after fasting as previously reported (16,19,20), whereas GHR mRNA level is not completely parallel with IGF-I level at least during an initial period after the start of fasting. In experiment 2, the effect of bGH administration on hepatic GHR/GHBP mRNA levels as well as plasma IGF-I levels were examined in the fasted and fed rats. The decreased IGF-I level and body weight by fasting was partially recovered by GH administration at the first day of fasting (TABLE 1). It is unlikely, therefore, that the decrease in plasma IGF-I levels after 1 day of fasting is caused by GH resistance. Since fasting causes suppression of GH secretion in rats (21), the decrease in plasma IGF-I levels 1 day after fasting may be attributed to the reduced GH release. In rats fasted for 5 days, exogenous GH administration failed to increase plasma IGF-I level, indicating development of resistance to GH. Refractoriness to GH in sustained fasting rats may be explained by the decrease in the number of GHR, since hepatic GHR mRNA level was significantly decreased and GH binding to liver membrane was also reduced in prolonged fasting state (16,17,22). However, post-receptor impairment in the fasting state cannot be excluded, since J.P. Thissen et al. have reported that plasma IGF-I responded poorly to GH injection in protein-restricted rats even after the decrease in GH binding to liver membranes was recovered by continuous infusion of GH(23). It is also reported that IGF-I synthesis is more sensitive to nutritional factors than to GH in steer(24). GH administration differentially affects hepatic GHR mRNA levels in fed and fasted rats.

In fed rats, GH administration caused a significant increase in GHR mRNA levels after 1 day protocol but failed to alter after 5 days protocol. In agreement with the change of GHR mRNA levels in fed rats, it is reported that repeated injections of GH caused a small increase in GH bindings to hepatic membrane in lambs on high nutrition(25). This GH-induced increase in GHR mRNA level might be explained not only by direct action of GH but also by autocrine or paracrine action of IGF-I or other local factors induced by GH. In fasted rats, however, GH administration failed to change GHR mRNA levels after 1 day fasting but caused a dramatic decrease in GHR mRNA levels after 5 days fasting. The mechanism by which the decline of GHR mRNA by GH is more exaggerated in fasting state remains to be solved. As mentioned previously, fasting causes a decrease in GH release in rats, and the regulation by GH of hepatic GHR mRNA expression may be very sensitive to exogenous GH in the GH-deficient state. Besides, Maiter D. et al. have reported that no remarkable change in liver concentration of GHR mRNAs was seen after both repeated injection and continuous infusion of GH in hypophysectomized rats(26).

In our studies, GH administration failed to change hepatic GHBP mRNA levels in fasted as well as fed rats. Maiter et al. also showed no apparent change of GHBP mRNA level by intermittent administration of GH in hypophysectomized rats(26). However, the possibility that GHBP mRNA expression is regulated by GH cannot be excluded, since a different way of GH administration could affect GHBP mRNA levels so that continuous infusion of GH increased liver GHBP mRNA levels in hypophysectomized rats(26). The effect of sustained serum GH elevation on GHBP mRNAs has also been studied in transgenic mice expressing a metallothionein 1(MT)-oGH fusion

gene(27), in which both the 3.9 kb GHR mRNA and the 1.2 kb band probably corresponding to mouse GHBP mRNA, were coordinately increased in the liver.

It is also important whether the regulation of GHR mRNA or GHBP mRNA is tissue specific. Transient elevation of GHBP mRNA by fasting was also observed not only in liver but also in muscle and heart, but not in fat tissue. The expression of GHR mRNA was too low to monitor the regulation. The previous studies revealed a variation in the contents of both mRNAs in liver, kidney, lung and ileum during somatic development (7). Another study has also demonstrated the increase in GHR mRNA by hypophysectomy in liver and muscle but decrease in fat tissue(28).

In summary, we demonstrated here the separate regulation of hepatic GHR mRNA and GHBP mRNA by fasting and GH administration and the tissue specific regulation by fasting of GHBP mRNA in rats. It is probable that the serum level of GHBP might not simply reflect the hepatic level of GHR, although it is still unknown whether the regulation of GHR mRNA and GHBP mRNA is different among species.

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