Pituitary control of lipoprotein and bile acid metabolism in male rats: growth hormone effects are not mediated by prolactin

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Matsasconi, Manuela, Bo Angelin, and Mats Rudling. Pituitary control of lipoprotein and bile acid metabolism in male rats: growth hormone effects are not mediated by prolactin. Am J Physiol Endocrinol Metab 287: E114–E119, 2004. First published March 16, 2004; 10.1152/ajpendo.00564.2003.—Previous studies have established that growth hormone (GH) has many important effects on the regulation of cholesterol and lipoprotein metabolism. However, human GH (hGH) can also bind to prolactin receptors, eliciting prolactin receptor-mediated effects. In this study, we evaluated whether hGH can exert such responses in currently used animal models and whether prolactin affects lipoprotein and/or hepatic cholesterol metabolism. Normal and hypophysectomized (Hx) male rats were given either hGH or bovine GH, the latter unable to bind to the prolactin receptor. The hormones were continuously infused by use of subcutaneous osmotic mini-pumps for 7 days; blood and livers were collected after euthanasia. Both hormones stimulated hepatic LDL receptor expression and bile acid synthesis to a similar extent and normalized the altered plasma lipoprotein pattern in Hx rats. Prolactin, injected twice daily to Hx male rats, did not exert any effects on the plasma lipoprotein pattern or on cholesterol metabolism. We conclude that previously established effects of hGH on cholesterol metabolism are not mediated by prolactin in male rats.

Plasma cholesterol; low-density lipoprotein receptor; cholesterol-7α-hydroxylase

THE PITUITARY EXERTS important regulatory effects on cholesterol and lipid metabolism in vivo in both animals and humans through the secretion of growth hormone (GH), adrenocorticotropic hormone (ACTH), and thyrotropin-releasing hormone (TSH) (8, 9, 12, 20, 25, 30, 33, 35). After hypophysectomy (Hx) of rats, the content of cholesterol is shifted from high-density lipoproteins (HDL) to low-density lipoproteins (LDL) (30, 34). Concomitantly, the activity of the rate-limiting enzyme in bile acid synthesis, cholesterol-7α-hydroxylase (cyp7a1), and fecal bile acid excretion are reduced (35). Hx of rats also results in a severe loss of resistance to dietary cholesterol (30) and a blunted stimulatory response in hepatic LDL receptor (LDLR) expression after cholesterol feeding (30, 34) or high-dose estrogen treatment (33). All of these metabolic changes seen in Hx rats can be normalized by the infusion of human GH (hGH) (30, 33–35). These effects of GH appear to be direct, since they cannot be reproduced by the infusion of IGF-I instead of hGH (34). When administered to normal rats, hGH induces hepatic LDL-Rs and cyp7a1 activity (27). However, both hGH and IGF-I induce LDL-Rs in cultured human hepatoma cells (26), showing that in vitro models do not adequately mirror the situation in vivo here.

In our previous experiments, hGH was administered as continuous infusions to normal and Hx male rats (17, 27, 30, 33–35), thus mimicking the female pattern of GH secretion observed in intact animals. It has previously been shown that, in addition to binding to GH receptors, hGH can also bind to prolactin receptors (4–6, 10, 23, 24, 29). It is known that normal male rats have low expression of hepatic prolactin receptors (29). However, previous studies have demonstrated that prolactin receptors can be induced after treatment that increases prolactin levels in plasma (1, 24). Therefore, it is plausible that prolactin may exert metabolic effects in male rats as it does in female rats in which hepatic prolactin receptors are abundant (1, 4, 6, 18, 24, 28). In addition, treatment of male rats with a continuous infusion of hGH stimulates prolactin receptors in male rat liver (1, 4, 18, 24, 28). In rat studies, bovine GH (bGH) is frequently employed because of its previously assigned low or absent lactogenic effects (23, 25). The responses obtained after treatment of male rats with hGH could thus hypothetically be due to such lactogenic effects of hGH. Indeed, recent data indicate that prolactin exerts effects on bile acid transport (6, 7, 14, 15, 21). To test the hypothesis that the metabolic responses after hGH treatment might be due to lactogenic effects of hGH, we compared effects on cholesterol metabolism after treatment of normal and Hx male rats with bGH or hGH. In addition, we investigated the effects of prolactin administration in Hx rats.

Our data show that all of the effects obtained after hGH treatment can be elicited by bGH, but not by prolactin. This indicates that the metabolic responses after infusion with hGH are not likely due to the lactogenic effects of this hormone and that prolactin is not involved in the regulation of hepatic cholesterol metabolism by the pituitary.

MATERIALS AND METHODS

Animals

Altogether, 74 male Sprague-Dawley rats (41 intact and 33 Hx; Taconic A & B, Ry, Denmark) were used in three separate experiments. Animals were kept under standardized conditions with free access to water and chow; lights were on between 6 AM and 6 PM. Hx was performed on 200-g animals, and body weight was monitored to verify failure to gain weight after surgery. Rats were killed by decapitation during isoflurane anesthesia, and trunk blood was collected. Livers were snap frozen in liquid nitrogen, and samples were

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stored at −80°C. The study was approved by the institutional animal care and use committee.

**Hormone Treatment**

We obtained bGH through Dr. A. F. Parlow [National Hormone and Peptide Program (NHPP), Harbor-UCLA Medical Center, Torrance, CA], and hGH was from Pharmacia (Stockholm, Sweden). The hormones were infused at the indicated doses for 7 days with osmotic minipumps (Alzet model 2001, Alza, Palo Alto, CA). The pumps were implanted subcutaneously in the dorsal region under light isoflurane inhalation, as previously described (27). Ovine prolactin (oPRL), also obtained from Dr. A. F. Parlow (NHPP), was administered subcutaneously by two daily injections for 7 days at 0.5 or 1.5 mg·kg⁻¹·day⁻¹. Control animals were sham-operated (pump experiment) or injected subcutaneously with vehicle. The vehicle for oPRL was 0.03 M NaHCO₃ in 0.15 M NaCl, pH 8.5. Solubilized oPRL was kept at +4°C. The injected volume was 200 μl.

**Lipid Assays**

**Cholesterol and triglycerides.** Total cholesterol and triglycerides were determined in 50-μl plasma aliquots from all individual animals by use of a Monarch Automated Analyzer (ILS Laboratories Scandinavia, Stockholm, Sweden).

**Size fractionation of plasma lipoproteins.** Size fractionation was performed by a fast performance liquid chromatography (FPLC) system with a micro FPLC column (30 × 0.32-cm Superose 6B purchased from Amersham Pharmacia, Upplands Väsby, Sweden) coupled to a system for online separation and subsequent detection of cholesterol, as previously described (16). Ten microliters of plasma from every individual animal were analyzed.

7α-Hydroxy-4-cholesten-3-one (C4), which strongly reflects bile acid synthesis and the enzymatic activity of Cyp7a1 (2, 3, 13, 36, 37), was assayed by HPLC using 200 μl of plasma. Samples from each animal were diluted with saline, an internal standard was added, and samples were extracted, eluted, dried, and dissolved in acetonitrile for HPLC analysis, as described in detail elsewhere (13).

**Expression of hepatic LDL-Rs and β-actin.** Hepatic membranes were prepared from liver tissue from individual animals or from pools from each group, as previously described (33). The membrane proteins were separated on 6% SDS-PAGE under nonreduced conditions from each group, as previously described (33). The membrane proteins were separated on 10% Tris-HCl precast gels and transferred to nitrocellulose filters. Ligand blots were performed to assess the expression of LDL-R using 125I-labeled rabbit β-migrating VLDL. The LDL-R bands (~120 kDa) were visualized using a Fuji Bioimaging BAS 1800 Analyzer (Fuji Photofilm, Tokyo, Japan) and quantified using Image Gauge Software (Science Lab 98, version 3.12, Fuji Photofilm). Expression of β-actin (~45 kDa) was determined by Western blot with a Criterion system (Bio-Rad, Hercules, CA): 75 and 200 μg of hepatic membrane proteins were separated on 10% Tris-HCl precast gels and transferred to nitrocellulose filters under reduced conditions (4% 2-mercaptoethanol; Merck, Darmstadt, Germany). The filters were then incubated overnight with a primary antibody against β-actin (Ab8227; rabbit polyclonal, diluted 1:10,000), washed, and incubated for 2 h with a peroxidase-conjugated goat anti-rabbit IgG (Ab6721; diluted 1:5,000). Both antibodies were purchased from Abcam (Cambridge, UK). Chemiluminescence substrate (Supersignal; Pierce, Rockford, IL) was added, and the filters were exposed to Cronex 5 X-ray films (Agfa-Gevaert, Mortsel, Belgium). Chemiluminescence was determined with a LAS 1000 plus luminescent image analyzer (Fuji Photofilm). Quantification of data was performed using Image Gauge software (Science Lab 98 version 3.12, Fuji Photofilm).

**Statistics**

Data are presented as means ± SE. The significance of differences between groups was tested by one-way ANOVA with Dunnett’s test.
when multiple groups were compared. To stabilize the variances, data were log transformed when the assumption for no correlation between means and variances was violated.

RESULTS AND DISCUSSION

Previous studies have suggested that hGH, but not bGH, can exert lactogenic effects in the rat because it can bind to prolactin receptors (5, 24, 29). It has been reported that hGH can induce prolactin receptors in the liver (23, 29), as does prolactin itself (1, 28). Because of such findings, bGH has commonly been used in animal experiments (8, 11, 12, 20, 25). To the best of our knowledge, the important question of whether metabolic responses obtained in vivo after the administration of hGH can actually be attributed to lactogenic effects of this hormone has not previously been addressed.

To answer this question, we studied some previously established metabolic responses to GH on cholesterol and bile acid metabolism in frequently employed animal models (30, 33, 35). We first compared the effects following administration of hGH and bGH to normal rats. Groups of intact male rats received continuous infusions of bGH or hGH (1 and 3 mg·kg\(^{-1}\)·day\(^{-1}\)) for 1 wk. The control animals were sham operated. It was found that both hGH and bGH increased plasma total cholesterol levels by \(\sim 25\%\) (Fig. 1A). Plasma total triglycerides were not altered. Plasma lipoprotein profiles obtained by micro-FPLC (Fig. 1B) revealed that the increase in plasma cholesterol was mainly within large HDL particles, in agreement with previous results (27, 35).

Both hGH and bGH treatments induced hepatic LDL-R protein expression (Fig. 1C). To compare the stimulatory effects of hGH and bGH on cyp7a1 activity (27, 32, 35), we analyzed the plasma levels of C4, a bile acid metabolite that strongly reflects the enzymatic activity of hepatic cyp7a1 and bile acid synthesis (2, 3, 13, 36, 37). Both bGH and hGH increased the plasma C4 levels by \(\sim 60\%\) (Fig. 1D).

Having established that bGH had essentially similar effects to hGH on plasma cholesterol, hepatic LDL-R, and plasma C4 levels in intact Sprague-Dawley rats, we then compared the effects of the two hormones in Hx rats. Total plasma cholesterol was increased by 20% after Hx, and treatment of Hx rats with either bGH or hGH (1.5 mg·kg\(^{-1}\)·day\(^{-1}\)) for 1 wk further increased total plasma cholesterol to a similar extent (Fig. 2A). Total plasma triglycerides were reduced by \(\sim 30\%\) after Hx and were not altered by GH treatment. Size fractionation of plasma lipoproteins typically revealed a strong increase in LDL cholesterol in Hx rats, whereas HDL cholesterol was reduced (30, 34, 35) (Fig. 2B). Treatment with either bGH or hGH shifted plasma cholesterol from LDL to HDL, in line with previous studies (30, 34, 35). The effect of bGH on the plasma cholesterol profile was somewhat less pronounced than that of hGH.
We then compared the effects of hGH and bGH on hepatic LDL-R expression by ligand blot. Because previous studies by Keller and Taylor (19) showed that total protein and RNA levels are significantly reduced in Hx animals, we also corrected our results to a “housekeeping gene.” Assay of β-actin showed 40% higher (P < 0.001) levels in liver membranes from Hx rats after correction for total protein (not shown). Three separate ligand blots were then run to assay the LDL-R to include all 24 animals at two protein concentrations per animal. The calculated final results from these three blots are presented in Fig. 2C, with and without correction for β-actin. In this experiment, it was found that both bGH and, to a lesser extent, hGH could induce hepatic LDL-R in Hx rats. Although the stimulation with hGH did not reach statistical significance in this experiment, the stimulatory response of hGH on hepatic LDL-Rs in Hx rats has previously been clearly shown (30, 34).

After correction for β-actin, Hx showed a stronger impact on LDL-R expression.

Plasma C4 levels were reduced by 80% (Fig. 2D) in Hx animals, in agreement with previous experiments in which cyp7a1 activity was determined (35). Treatment with bGH resulted in a 2.5-fold increase of C4 levels, and treatment with hGH showed a 4-fold increase of C4 levels.

Because bGH and hGH exerted similar effects in both normal and Hx rats, the results suggest that the responses obtained after hGH administration are not likely due to prolactin-mediated effects of hGH. Nevertheless, we then studied the effects of administration of oPRL in the same animal model. Hx rats were injected twice daily with one of two doses of oPRL (0.5 and 1.5 mg·kg⁻¹·day⁻¹, respectively) for 7 days (Fig. 3). It was found that both groups of Hx rats receiving oPRL significantly increased in body weight compared with Hx controls. The increase was ±4.0 ± 0.7 g (P < 0.05) and ±8.0 ± 0.7 g (P < 0.05) for the low and the high doses, respectively, of oPRL. A significant weight gain was present already after 5 days of treatment at both doses. This finding, together with the previously reported fact that rats respond to PRL treatment with an increase in body weight (22), suggests that the oPRL preparation and way of administration were adequate in our experiment. Total plasma cholesterol and triglyceride levels in Hx rats were not significantly altered after prolactin treatment (Fig. 3A). Analysis of the plasma lipoprotein profiles revealed that oPRL treatment was without effect on the typically altered lipoprotein pattern of Hx rats (Fig. 3B).

Assay of hepatic LDL-R expression again confirmed that LDL-Rs are reduced in Hx rats (31, 35). Treatment with prolactin did not induce LDL-Rs; if anything, a slight reduction was obtained (Fig. 3C). Correction of the data for β-actin further confirmed the inability of oPRL to induce LDL-Rs in Hx male rats (Fig. 3C). Finally, we analyzed the plasma levels of C4 (Fig. 3D). Again, plasma C4 levels in Hx rats were reduced by ~70% compared with intact controls, whereas

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Fig. 3. Treatment of Hx rats for 7 days with ovine prolactin (oPRL). Indicated amounts of oPRL were injected sc twice daily. Results are expressed as means ± SE. Statistical comparison was performed for PRL-treated animals using untreated Hx rats as controls. There were 5 animals/group. A: total plasma cholesterol (filled bars) and triglycerides (open bars). B: lipoprotein profiles (cholesterol) after separation of plasma by micro-FPLC. Each line shows the average from all 6 individuals in every group as shown. C: hepatic LDL-R expression by ligand blot using rabbit 125I-β-VLDL. Four samples (75-μg liver membranes) were assayed per group except for low-dose oPRL, for which 3 samples were used. Quantified values of ligand blot (% of controls) are shown as means ± SE. D: plasma levels of C4 (ng/ml). Assays for C4 were made on all individual animals.
oPRL did not increase plasma C4 levels; if anything, a slight reduction was seen. Thus none of the responses obtained by hGH or bGH in male Hx rats could be elicited in vivo after treatment with prolactin. In line with these findings, previous studies on human hepatoma cells incubated in vitro with PRL showed no effects on LDL-Rs (26).

In conclusion, we have shown that important regulatory events in cholesterol and bile acid metabolism can also be elicited with bGH, as with hGH, but not by oPRL administration, strongly suggesting that these metabolic effects are not due to lactogenic responses in this animal model.

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