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Microarray Analysis of the *in Vivo* Effects of Hypophysectomy and Growth Hormone Treatment on Gene Expression in the Rat*

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ABSTRACT

Complementary DNA microarrays containing 3000 different rat genes were used to study the consequences of severe hormonal deficiency (hypophysectomy) on the gene expression patterns in heart, liver, and kidney. Hybridization signals were seen from a majority of the arrayed complementary DNAs; nonetheless, tissue-specific expression patterns could be delineated. Hypophysectomy affected the expression of genes involved in a variety of cellular functions. Between 16–29% of the detected transcripts from each tissue changed expression level as a reaction to this condition. Chronic treatment of hypophysectomized animals with human GH also caused significant changes in gene expression patterns. The study confirms previous knowledge concerning certain gene expression changes in the abovementioned situations and provides new information regarding hypophysectomy and chronic human GH effects in the rat. Furthermore, we have identified several new genes that respond to GH treatment. Our results represent a first step toward a more global understanding of gene expression changes in states of hormonal deficiency. (*Endocrinology* **142:** 3163–3176, 2001)

THE PITUITARY GLAND is the main regulator of the endocrine system (1). It exerts its actions through the secretion of trophic hormones that regulate the functions of multiple endocrine glands. Virtually every tissue in the body is targeted for hormone action and thus is influenced by the failure of different endocrine systems that follow hypophysectomy. The failure in growth and reproduction that grossly characterize hypophysectomy is paralleled by numerous other changes of a tissue-specific or metabolic nature. Hypophysectomized (Hx) animals have been widely used as models to investigate biological consequences of severe hormonal deficiency as well as to clarify individual hormonal actions (2, 3).

GH is primarily synthesized in the pituitary gland. It is the main regulator of longitudinal growth and exerts a multitude of effects on several tissues. In liver, GH regulates lipid, protein, and carbohydrate metabolism (4, 5). It is responsible for sex-specific regulation of the metabolism of xenobiotic compounds (6) and regulates the expression of multiple liver-specific secretory proteins, including insulin-like growth factor I (IGF-I), a key regulator of body growth (7). GH also regulates cardiovascular function through effects in both heart and peripheral vasculature, and it induces cardiac growth and increases contractility in GH-deficient individuals and animal models (8). In kidney, the GH/IGF-I axis may be a cause of the glomerular and proximal tubular hypertrophy that occurs in hypersomatotropic states and during the development of diabetic kidney disease (9). IGF-I has also been reported to be involved in compensatory renal hypertrophy, and renal regeneration after acute ischemic injury (10). Nevertheless, the molecular mechanisms responsible for these actions remain unknown in many cases.

Technological developments have made it possible to monitor changes in gene expression of multiple transcripts and thereby generate large gene expression profiles. DNA microarrays can be used to simultaneously monitor several thousand different transcripts (11). We believe that the identification of gene expression patterns induced by specific hormones will provide insights into the molecular events underlying their diverse and tissue-specific actions.

In the present study we have used DNA microarrays containing 3000 different rat complementary DNA (cDNA) clones to investigate the state of hypophysectomy in three rat tissues, namely liver, kidney, and heart. Common and tissue-specific changes in gene expression were analyzed. Pituitary-dependent transcripts were classified according to biochemical function. In the last part of this study we examined the effects of 3 weeks of continuous human GH (hGH) infusion into Hx animals. Attempts were made to correlate the newly described variations in the expression patterns to the phys-

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iological changes induced by hypophysectomy and GH treatment.

Materials and Methods

RNA preparation and messenger RNA (mRNA) isolation

Young male Sprague Dawley rats, Hx at 6 weeks of age, and age-matched intact rats (Möllegaards Breeding Center Ltd., Ejby, Denmark) were maintained under standardized conditions of light and temperature, with free access to food and water for 3 weeks. Some of the Hx rats were treated with recombinant human GH (hGH) by continuous infusion from osmotic minipumps (model 2004, Alza Corp., Mountain View, CA). hGH (a gift from Pharmacia & Upjohn, Inc., Piscataway, NJ) was administered to animals at a daily dose of 0.34 μg/g BW. After 3 weeks of treatment, the rats were killed, and tissues were collected, frozen in liquid nitrogen, and stored at −80 C. The animal experiments were approved by the local ethical committee.

Total RNA was isolated using TRIzol (Life Technologies, Inc., Gaithersburg, MD), according to the protocol supplied by the manufacturer. The quality of the RNA samples was ascertained on a denaturing agarose gel. Equal amounts of total RNA from four animals in the same experimental group were pooled before mRNA purification. mRNA was purified from 1 mg total RNA using 35 mg oligo(deoxythymidine)-cellulose (Amersham Pharmacia Biotech, Uppsala, Sweden) equilibrated in a ribonuclease (RNase)-free binding buffer containing 20 mM Tris-HCl, 0.5 M NaCl, and 1 mM EDTA. Polyadenylated [poly(A)+] RNA was bound to the cellulose and washed four times using the same buffer, then washed once in a low salt buffer (0.1 M NaCl) and eluted twice in warm elution buffer (20 mM Tris-HCl and 1 mM EDTA).

Probe labeling and purification

Two to 4 μg poly(A)$^+$ mRNA were used from each group of animals for each experiment. Labeled cDNA was produced by a random primed RT reaction in the presence of Cy-labeled nucleotides (12). Mouse Cot1 DNA (10 μg; Life Technologies, Inc.) was added to each reaction, and the probes were subsequently purified as previously described (12). The hybridization buffer consisted of 5 × SSC (standard saline citrate)/0.2% SDS, 10 μg poly(A) RNA, and 10 μg yeast transfer RNA. The probe was added at a final volume of 15 μl to the array, a plastic 22 × 22-mm coverslip (Grace Bio-Labs, Bend, OR) was placed on top, and the array was put in a sealed hybridization chamber (Corning, Inc., Corning, NY). Hybridization took place at 65 C for 15–18 h. The array was then washed (12) and immediately scanned using the GMS 418 scanner (Affymetrix, Santa Clara, CA).

Rat cDNA clone collection

We collected approximately 3000 rat cDNA clones, selected from the TIGR Rat Gene Index (www.tigr.org) and from earlier subtractive cloning experiments (13). Fifty percent of these clones represent previously characterized rat genes or mouse/human orthologs, and the rest are identified by expressed sequence tags and have no significant homology to sequences contained in public databases.

Generation of cDNA microarrays

Bacterial colonies were grown overnight in 1.5 ml Luria Bertoni medium in 96-well plates (Beckman Coulter, Inc., Palo Alto, CA), and plasmid minipreparations were followed by a PCR amplification of the inserts using vector-specific primers T3 (5′AATTAACCCTCACTAAAGGG-3′) and T7 (5′-GTAATACGACTCACTATAGGGC-3′). The amplified inserts, each produced by two pooled 100-μl PCR reactions, were purified by ethanol precipitation and resuspended in 40 μl 3 × SSC, and purity was checked on an agarose gel. Approximately 10% of the cDNA clones rendered more than one band after PCR amplification. CMT GAPS amino silane-coated slides (Corning, Inc.) were used as adhesive surface for printing using a GMS 417 arrayer (Affymetrix). The slides were postprocessed as described previously (12) and stored in a dust-free dark box until hybridization.

Chip design and interpretation

Individual cDNAs were spotted once on the glass surface, and selected cDNAs were spotted twice at different locations on the chip to serve as reproducibility controls. The hybridizations were repeated using new sets of labeled targets, acting as an additional control of reproducibility. The cut-off for selecting a gene as up- or down-regulated was set at 1.5 based on comparison of the results from the chip analysis with independent validation using RNase protection assay (see *Results*). These results show that Cy5/Cy3 ratios of approximately 1.5 correspond to higher ratios when calculated using RNase protection analysis. This cut-off is also supported by independent findings that show regulation by GH of the expression of genes such as GLUT-1 (14), fibrinogen (15), MHC-β (16), CYP2C7, ferritin, transferrin (17), and CYP3A2 (18). Furthermore, only changes in gene expression that were reproducible and higher than the cut-off in at least two independent experiments are presented. Data from clones rendering more than one band after PCR amplification were removed from the results because they represent more than one gene. All genes mentioned here by name have been sequence verified by single pass 3′-sequencing.

Image analysis

Image analysis was performed using the ScanAlyze software (available at http://rana.stanford.edu/software). The signal from each spot was calculated as the average intensity for each channel. Automatic and manual flagging were used to localize absent or very weak spots (<1.6 times above background), which were excluded from analysis. Normalization between the two fluorescent images was performed, as previously described (19), by applying a scaling factor to all intensities measured for the Cy5 channel so that the mean log (Cy5/Cy3) for the subset of spots that were not flagged was 0.

Solution hybridization/RNase protection analysis

Total nucleic acids (tNA) were isolated by homogenization of tissue specimens, using a Polytron PT-2000 (Kinematica AG, Lucerne, Switzerland). Digestion of samples with proteinase K (Merck, Mannheim, Germany) and subsequent extraction with chloroform and phenol were described previously (20). mRNA levels corresponding to the expression of individual clones [stearyl coenzyme A (CoA) desaturase 1 (SCD1), meprin A, and atrial natriuretic peptide] were measured in tNA samples using a solution hybridization/RNase protection assay as described previously (13). Transcript-specific ^{35}S-labeled complementary RNA probes were transcribed *in vitro* from the respective cDNA vector construct, according to the method of Melton *et al.* (21). All probes were cloned into the pT3T7Pac vector. Reagents for *in vitro* transcription were obtained from Promega Corp. (Madison, WI). The concentration of nucleic acids in tNA samples was measured spectrophotometrically. To permit accurate comparison of specific mRNA levels between different hormonal statuses, the samples of interest were always analyzed at the same assay occasion. The DNA content of the tNA samples was measured with the DyNA Quant 200 system (Hoefer Scientific, San Francisco, CA) according to the supplied protocol. The Hoechst dye was purchased from Polysciences, Inc. (Warrington, PA). Samples were analyzed in triplicate, and the results are expressed as counts per min of specific mRNA per μg DNA.

Results

Effects of hypophysectomy on gene expression

Hypophysectomy in combination with hormonal replacement in rats has been classically used to study hormonal regulation of different physiological processes. DNA microarray technology provides a novel platform to access the physiological consequences of this experimental paradigm by analyzing global changes in gene expression. We have studied the effects of hypophysectomy on gene expression in three different tissues: liver, kidney, and heart. Moreover, the effects of chronic hGH replacement therapy on Hx animals were also analyzed in the same tissues.

The microarrays were hybridized with fluorescent probes derived from poly(A)$^+$ mRNA isolated from liver, heart, and kidney. Each hybridization compared Cy3-labeled cDNA, reverse transcribed from a pool of mRNA isolated from four 12-week-old, nontreated, male rats (normal), with Cy5-labeled cDNA produced from a pool of mRNA isolated from four age-matched animals that had been hypophysectomized for 6 weeks. In another set of experiments, Cy3-labeled cDNA derived from tissues of 12-week-old Hx animals was compared with Cy5-labeled cDNA from age-matched Hx animals that had been continuously treated with hGH for 3 weeks. Hybridizations were performed in duplicate, and only changes reproducible across independent replicates were considered. The hybridized fluorescent targets were detected, and signal intensities for the two fluorescent images were normalized, so that the mean log (Cy5/Cy3) of all detectable spots was zero (19).

An important consideration is that hypophysectomy causes a marked decrease in RNA content in multiple tissues (22). In our hands, and in agreement with previous results, the content of RNA per cell is reduced by approximately 40% in liver by hypophysectomy, and the fraction of mRNA in the total pool follows this pattern (23). However, equal amounts of mRNA from tissues derived from Hx and normal rats were used to guarantee proper array quantification and data processing (12). By using this normalization strategy, under- and overrepresented transcribed genes could be identified in the mRNA pools (Tables 2 and 3), whereas the ratios measured for the vast majority of genes, including housekeeping genes such as β-actin and histones, were approximately 1 in all three tissues.

Hybridization data are summarized in Fig. 1. Of the 3000 arrayed clones, about 720 were detectable (signal 1.6-fold above background) in liver, 1600 in heart, and 985 in kidney. Some of these were expressed in more than 1 tissue, whereas others could only be detected in 1 of the tissues analyzed (Fig. 1A). Of the total detectable elements, 82 clones were overexpressed (at least 1.5-fold increase), and 127 were underexpressed (at least 1.5-fold decrease) in liver from Hx animals compared with normal liver. This represents 29% of the detectable transcripts in this tissue. In the kidney samples, 91 genes were overexpressed, and 136 were underexpressed in Hx animals. This number represents 23% of the total number of detected transcripts. In heart, 125 genes were overexpressed, and 141 were underexpressed in tissues from Hx rats compared with normal tissues. The percentage of regulated genes thus was lower in heart (16%) than in the other tissues. The identities of the known genes and their putative functions are listed in Tables 2 and 3.

GH regulation of gene expression in liver, kidney and heart of the hypophysectomized rat

GH treatment of Hx rats restores growth and has major effects on metabolism. Using this model, a significant amount of physiological information on GH action has been collected (3). Therefore, we compared the effects of GH treatment (3 weeks of continuous infusion of hGH) on the gene expression patterns in three tissues of Hx animals. Table 4 shows the effects of chronic GH treatment on gene expression (only genes with a putative identity are listed) in kidney, liver, and heart of Hx animals. GH regulates the expression of about 90 different genes in the tissues analyzed. In liver, 28 genes were overexpressed after GH treatment, and 30 were underexpressed. In kidney, 7 were overexpressed, and 9 were underexpressed, whereas in heart 22 were overexpressed, and 10 were underexpressed (Fig. 1B).

GH treatment alone was unable to restore the normal pattern of gene expression in the Hx animals. This was expected, as hypophysectomy causes severe hormonal deficiency that goes beyond GH insufficiency. Many of the physiological effects of GH in this animal model require combined hormonal replacement with thyroid hormone and glucocorticoids. Nevertheless, GH is expected to restore the expression of certain genes independently of other hormones. For example, GH is known to induce the expression of CYP2C12 and CYP2C7 and decrease the expression of CYP3A2 and carbonic anhydrase III in the liver of Hx male rats (6). Our microarray results confirmed these findings and identified several novel GH-regulated genes, some of which were highly enriched in one of the tissues analyzed. Three of those genes were selected, and their expression levels were measured using RNase protection analysis. The results (Fig. 2) confirmed the array information and showed that the atrial natriuretic peptide, SCD1, and meprin A genes are indeed tissue enriched and that GH reverses the negative effect of hypophysectomy on their respective expression level. The analysis using RNase protection assay was then further extended to include four additional genes assigned as differentially regulated using DNA microarrays. As shown in Table 1, all seven selected transcripts were confirmed to be differentially expressed when analyzed by RNase protection assay. In terms of fold regulation, DNA chip analysis tended to underestimate differences compared with RNase protection assay.

Discussion

Effects of hypophysectomy on gene expression

Hypophysectomy is characterized by growth failure due to the loss of GH. Furthermore, gonadal, adrenal, and thyroid gland deficiencies result from the loss of trophic hormone secretion from the pituitary gland. This severe hormonal depletion alters protein, carbohydrate, and lipid metabolism and is coincident with a decline in appetite and food intake (3, 24). Hypophysectomy results in decreased fasting serum glucose concentration, impaired glucose tolerance, decreased insulin secretion, and increased insulin sensitivity (25, 26). Hx animals have a higher proportion of fat and a lower proportion of protein in the carcass and exhibit retarded release of fatty acids from adipose tissue in response to caloric restriction. The Hx rat also shows an increased protein catabolism with an increase in plasma urea concentration (3, 27).

Our analysis of the effects of hypophysectomy demonstrates that the expression of genes involved in virtually all cellular processes are affected, including metabolism, cell structure, protein turnover, and signal transduction (Fig. 1C). These wide effects are expected, as multiple cellular pro-

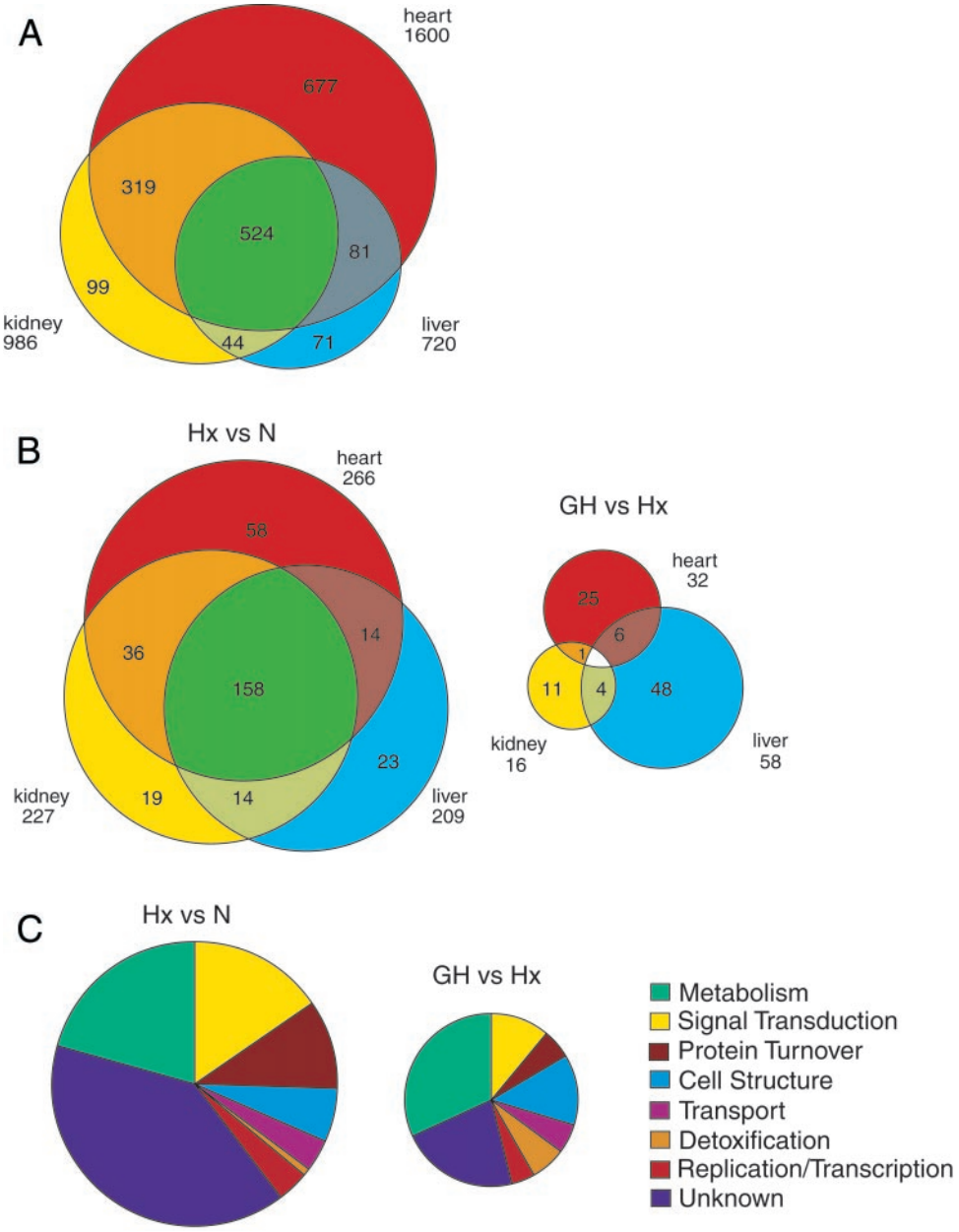

FIG. 1. Summary of results obtained in the microarray analysis of hypophysectomy and GH treatment in liver, kidney, and heart of the male rat. Each *circle* represents one organ, and the area is proportional to a number of transcripts. A, Number of mRNAs expressed in the different tissues (signal above background on microarray comparing normal to Hx tissue, reproduced in at least two independent experiments). B, Number of mRNAs differentially regulated in the comparison between normal and Hx tissues and in the comparison between Hx and GH-treated Hx liver, kidney, and heart. C, Functional classification of the differentially regulated transcripts.

cesses are regulated by traditional hormones as well as other trophic factors. Many of these changes in gene expression reflect the physiological changes observed in the Hx rat. Hypophysectomy causes a general decrease in the mRNA content of different tissues (23). This was reflected in the present study by the multiple genes underexpressed in tissues from Hx rats. Table 2 shows the genes that were more than 1.5-fold underexpressed after hypophysectomy.

Major effects were observed in genes involved in lipid and carbohydrate metabolism as well as in the mitochondrial respiratory chain. These findings reflect the metabolic changes observed in Hx animals, which have diminished metabolic rate, heat production, and oxygen consumption (3). Our observations that removal of the pituitary decreased the levels of several transcripts encoding various components of the electron transport chain, including cytochromes *b* and *c* and cytochrome *c* oxidase, are in agreement with previous studies of rat liver mitochondria. As early as 1972, Matsubara *et al.* reported that the respiratory cytochrome content of isolated mitochondria decreased in Hx animals in parallel with a decrease in respiratory activities (28). These changes have been attributed to an impaired function of the thyroid gland. Replacement therapy with thyroid hormone increases both respiration rates and mitochondrial mass (29).

Reduction in food intake, endogenous insulin secretion, and metabolic rate lead to diminished glucose utilization in multiple tissues in the Hx animal (26). This has been attributed to both a reduction in glucose transport and a progressive decrease in the activity of several enzymes involved in glucose oxidation (3, 26). When fed *ad libitum*, Hx rats maintain normal levels of blood glucose despite above normal rates of carbohydrate usage relative to other nutrients (27).

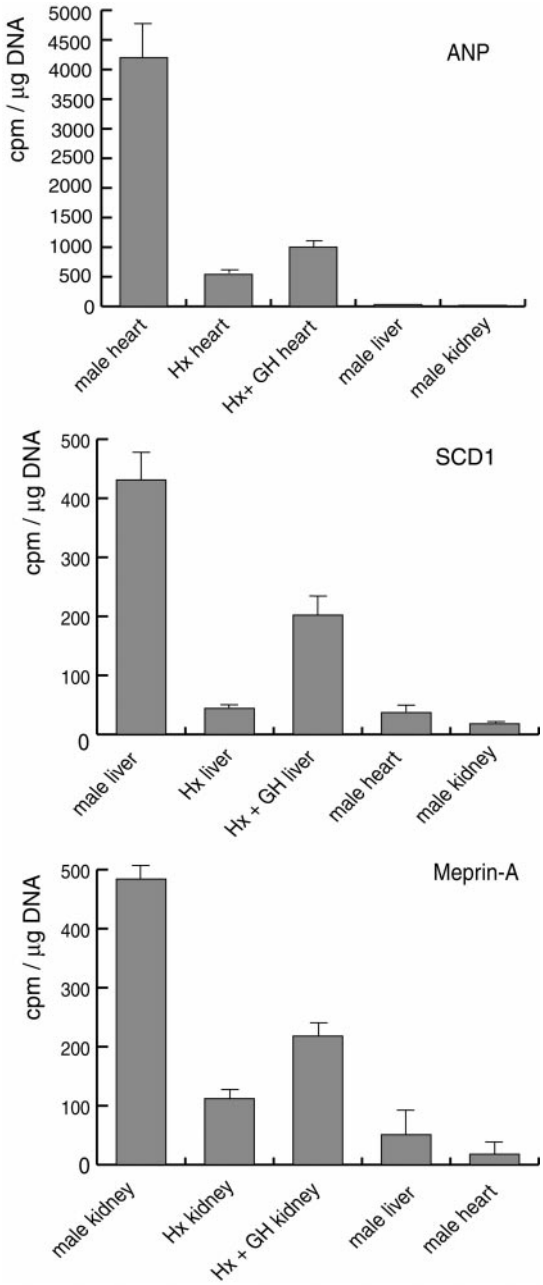

FIG. 2. RNase protection analysis of gene expression levels of meprin A, Stearyl-CoA desaturase 1 (SCD1), and atrial natriuretic peptide (ANP) in liver, kidney, and heart of normal, Hx, and GH-treated Hx male rats. The tissue-enriched expression patterns shown on the microarrays were confirmed.

TABLE 1. Comparison between Cy5/Cy3 ratio calculated after DNA microarray analysis and gene expression ratios calculated by RNase protection assay (RPA) for the following genes: atrial natriuretic peptide (ANP), stearyl-CoA desaturase 1 (SCD1), meprin A, farnesyl diphosphate synthetase (FDS), glutathione-*S*-transferase (GST), cytochrome b5, and insulin-like growth factor-binding protein 2 (IGFBP2)

Probe	Tissue	Expt	Ratio
Atrial natriuretic peptide	Heart	Hx *vs.* N (RPA)	0.10
		Hx *vs.* N (Cy5/Cy3)	0.48
		Hx + GH *vs.* Hx (RPA)	1.85
		Hx + GH *vs.* Hx (Cy5/Cy3)	1.79
Stearyl CoA desaturase	Liver	Hx *vs.* N (RPA)	0.10
		Hx *vs.* N (Cy5/Cy3)	0.07
		Hx + GH *vs.* Hx (RPA)	4.60
		Hx + GH *vs.* Hx (Cy5/Cy3)	2.65
Meprin A	Kidney	Hx *vs.* N (RPA)	0.23
		Hx *vs.* N (Cy5/Cy3)	0.64
		Hx + GH *vs.* Hx (RPA)	1.94
		Hx + GH *vs.* Hx (Cy5/Cy3)	1.51
Glutathione-*S*-transferase	Liver	Hx *vs.* N (RPA)	0.18
		Hx *vs.* N (Cy5/Cy3)	0.77
		Hx + GH *vs.* Hx (RPA)	3.53
		Hx + GH *vs.* Hx (Cy5/Cy3)	2.10
Farnesyl diphosphate synthetase	Liver	Hx *vs.* N (RPA)	0.15
		Hx *vs.* N (Cy5/Cy3)	0.63
		Hx + GH *vs.* Hx (RPA)	3.23
		Hx + GH *vs.* Hx (Cy5/Cy3)	1.44
Cytochrome b5	Liver	Hx *vs.* N (RPA)	0.26
		Hx *vs.* N (Cy5/Cy3)	0.80
		Hx + GH *vs.* Hx (RPA)	2.10
		Hx + GH *vs.* Hx (Cy5/Cy3)	1.77
IGFBP2	Liver	Hx *vs.* N (RPA)	11.00
		Hx *vs.* N (Cy5/Cy3)	6.69
		Hx + GH *vs.* Hx (RPA)	1.20
		Hx + GH *vs.* Hx (Cy5/Cy3)	1.39

The availability of fatty acids decreases, and the catabolism of amino acids increases (30). A striking difference was observed between heart and liver regarding the expression of genes encoding enzymes involved in fatty acid β-oxidation and pyruvate usage. Our experiments showed that the expression of pyruvate dehydrogenase kinase (isoenzyme 4), a negative regulator of the oxidation of pyruvate to acetyl-CoA (31), was down-regulated in heart by hypophysectomy, but not in liver. This is in agreement with a preferential utilization of carbohydrates over lipids as an energy source in the former tissue. In addition, the genes involved in β-oxidation of fatty acids (3-ketoacyl-CoA thiolase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 2,4-dienoyl-CoA reductase), an alternative source of acetyl-CoA for the Krebs cycle, were markedly reduced by hypophysectomy in heart. In liver, although pyruvate dehydrogenase kinase expression was less affected than in heart, the expression of lactate dehydrogenase was markedly reduced. As a result, there should be an increased availability of hepatic pyruvate for gluconeogenesis (27). In liver, the transcripts for the genes involved in β-oxidation were less affected than those in heart, which is in line with previous reports that showed no difference in hepatic respiratory CO_2 production after palmitate infusion in Hx *vs.* normal rats (32). Nevertheless, it is well known that hypophysectomy inhibits the hepatic production of ketone bodies during fasting. This has been attributed to decreased fatty acid mobilization rather than reduced capacity of the liver to produce ketone bodies (3). This decreased fatty acid mobilization from adipose tissue and liver may cause a reduced supply of fatty acids for β-oxidation in heart and muscle (33). This is in agreement with the previous idea that carbohydrates are the favored fuel in these tissues in Hx animals (34).

The liver is responsible for the maintenance of normal blood glucose levels (27). In fact, glycogen stores are reduced in muscle and liver after hypophysectomy, and liver glu-

TABLE 2. Effects of hypophysectomy, down-regulated genes

		Cy5/Cy3 ratio Hx/normal			Accession no.
		Liver	Heart	Kidney	
Carbohydrate/energy metabolism					
RGIAJ68	glucose-6-phosphatase, catalytic subunit	**0.27** ± 0.06		**0.53** ± 0.04	D78592
RGIAJ80	fructose-1,6-bisphosphatase	**0.60** ± 0.04		**0.44** ± 0.04	M86240
RGIBB52	aldolase B	**0.65** ± 0.07	1.09 ± 0.14	**0.61** ± 0.03	M10149
RGIAA27	enolase, α	0.77 ± 0.22	1.26 ± 0.22	**0.48** ± 0.09	X02610
RGIAE58	Phosphoglycerate mutase PGAM-B	**0.56** ± 0.04	**0.58** ± 0.11	**0.48** ± 0.05	S63233
RGIAN09	lactate dehydrogenase A	**0.47** ± 0.12	1.00 ± 0.15	1.05 ± 0.12	NM_017025
RGIBB11	pyruvate dehydrogenase kinase isoenzyme 4	1.14 ± 0.13	**0.39** ± 0.04		AF034577
RGIAK54	NADP-dependent isocitrate dehydrogenase	1.06 ± 0.10	0.88 ± 0.13	**0.40** ± 0.04	L35317
RGIAX15	transketolase	**0.53** ± 0.00	1.32 ± 0.01	1.08 ± 0.03	AW140979
RGIAF74	glycerol-3-phosphate dehydrogenase (mouse)	0.72 ± 0.02	**0.44** ± 0.04	0.84 ± 0.05	M25558
RGIAO35	aldo-keto reductase	**0.56** ± 0.07		0.80 ± 0.19	U68535
RGIAA84	Aldehyde reductase	0.79 ± 0.03	1.02 ± 0.24	**0.59** ± 0.03	D10854
RGIBA38	cytochrome *c* oxidase subunit II	**0.53** ± 0.08	**0.44** ± 0.11	**0.60** ± 0.10	M27315
RGIAX71	cytochrome *b*5, mitochondrial isoform	0.80 ± 0.15	**0.59** ± 0.04	0.86 ± 0.16	Y12517
RGIAX44	cytochrome *b* (mitochondrial gene)	**0.42** ± 0.11	**0.45** ± 0.05	**0.62** ± 0.04	AB033713
RNAB40	cytochrome *b*	**0.44** ± 0.10	**0.54** ± 0.05	0.69 ± 0.01	AF295545
RGIAQ01	cytochrome *b*558 α-subunit			**0.59** ± 0.02	677891
RGIAP60	cytochrome *c* pseudogene	**0.58** ± 0.09	**0.49** ± 0.11	**0.35** ± 0.08	K03238
RGIAG46	ATP synthase subunit 6 (mitochondrial gene)	**0.60** ± 0.17	**0.56** ± 0.01	**0.65** ± 0.03	AF115770
Fatty acid metabolism					
RGIAG12	3-ketoacyl-CoA thiolase[a]	1.21 ± 0.11	**0.40** ± 0.03	0.70 ± 0.05	P13437
RGIAE66	3-ketoacyl-CoA peroxisomal thiolase, nuclear gene	1.01 ± 0.08	**0.35** ± 0.09	0.76 ± 0.16	X05341
RGIAE70	Enoyl-CoA hydratase		**0.51** ± 0.06	1.16 ± 0.38	U08976
RGIAE78	3-hydroxyacyl-CoA dehydrogenase	0.94 ± 0.04	**0.56** ± 0.03	0.72 ± 0.08	AF095449
RGIAA64	2,4-dienoyl-CoA reductase	**2.05** ± 0.16	**0.55** ± 0.10	0.98 ± 0.30	D00569
RGIAE86	long-chain acyl-CoA synthetase (EC 6.2.1.3)	0.77 ± 0.14	**0.48** ± 0.12	0.99 ± 0.24	D90109
RGIAE62	stearyl-CoA desaturase 1	**0.07** ± 0.00			J02585
RGIAE80	Farnesyl pyrophosphate synthetase	**0.63** ± 0.02		0.83 ± 0.11	M34477
Intermediary metabolism					
RGIAE79	Ornithine aminotransferase	1.04 ± 0.23	1.09 ± 0.06	**0.59** ± 0.03	M11842
RGIAC87	Ornithine decarboxylase		1.21 ± 0.15	**0.49** ± 0.01	J04791
RGIAC56	Aromatic L-amino acid decarboxylase	1.05 ± 0.01	**2.26** ± 0.08	**0.63** ± 0.15	NM_012545
RGIAO17	glutamate dehydrogenase	**0.49** ± 0.06	**0.65** ± 0.06	0.72 ± 0.17	X14044
RGIAB17	Choline acetyltransferase		**0.56** ± 0.11	0.75 ± 0.03	NM_007760
RGIBA08	δ-aminolevulinate synthase	1.00 ± 0.08	**0.41** ± 0.09	0.83 ± 0.17	J04044
Signal transduction/trophic factors/receptors					
RGIAQ88	osteonectin	**0.58** ± 0.05	**0.61** ± 0.15	**0.64** ± 0.06	Y13714
RGIAK84	matrix GIa protein		**0.24** ± 0.04	**0.41** ± 0.01	NM_012862
RGIAB64	ADP-ribosylation factor 4	**0.66** ± 0.11	**0.64** ± 0.02	0.71 ± 0.01	M86705
RGIAJ70	aquaporin 8	**0.42** ± 0.09			AF007775
RGIAO22	hepatic fibrinogen/angiopoietin related protein (HARP)	**0.42** ± 0.02			AF169313
RGIAJ86	angiotensinogen	**0.44** ± 0.03			L00091
RGIAJ82	α2-HS-glycoprotein	**0.38** ± 0.03			NM_012898
RGIAV22	peroxisomal farnesylated protein	0.83 ± 0.17	**0.44** ± 0.09	0.96 ± 0.11	Y09049
RGIAG32	sarcoplasmic reticulum Ca^{2+}-ATPase		**0.49** ± 0.08	0.72 ± 0.01	X15635
RGIAR90	latent transform, growth factor-β-binding protein 4		**0.48** ± 0.04	1.05 ± 0.00	3327808
RGIAJ94	atrial natriuretic peptide		**0.48** ± 0.16		NM_012612
RGIAN93	PINCH protein (human)		**0.56** ± 0.07		AW140647
RGIAG40	Rab3B		**0.58** ± 0.05		Y14019
RGIAL96	growth arrest-specific gene gas1 (mouse)		**0.53** ± 0.14		AW140581
RGIAF84	profilin 1 (mouse)	0.86 ± 0.03	0.89 ± 0.17	**0.63** ± 0.01	NM_011072
RGIAC54	protein kinase PIM-3		1.38 ± 0.17	**0.55** ± 0.11	AF086624
RGIBB36	DAD-1 (defender against apoptotic cell death 1)		0.71 ± 0.14	**0.60** ± 0.06	AI013627
RGIBA44	calbindin D28			**0.34** ± 0.01	M27839
Cell structure					
RGIAQ36	fibronectin	**0.26** ± 0.01		0.91 ± 0.01	X15906
RGIAM23	collagen α1 type III	0.73 ± 0.09	**0.42** ± 0.06		X70369
RGIAQ90	extracellular matrix protein 1 (mouse)	0.82 ± 0.11	**0.47** ± 0.05		A57474
RGIAJ64	fibulin 5			**0.61** ± 0.06	NM_019153
RGIAX86	intestinal trefoil factor			**0.18** ± 0.07	NM_013042
RGIAJ60	bone proteoglycan II (decorin)		**0.66** ± 0.02	**1.68** ± 0.17	X59859
Protein folding					
RGIAB50	calreticulin[a]	**0.49** ± 0.03	0.82 ± 0.06	**0.66** ± 0.04	X53363
RGIAG81	DnaJ protein homolog (mouse)		1.09 ± 0.10	**0.42** ± 0.06	AB032401

TABLE 2. Continued

		Cy5/Cy3 ratio Hx/normal			Accession no.
		Liver	Heart	Kidney	
Protein degradation					
RGIAC92	protective protein for β-galactosidase (mouse)	0.68 ± 0.08	0.69 ± 0.05	**0.58** ± 0.06	AW914283
RGIAD82	proteasome subunit RC1	0.89 ± 0.12		**0.60** ± 0.01	D10729
RGIAD04	ER-60 protease (ER60)	**0.47** ± 0.06	0.85 ± 0.05	0.72 ± 0.01	NM_017319
RGIAV42	meprin A β-subunit			**0.64** ± 0.21	NM_013183
Detoxification					
RGIAJ76	CYP2C7	**0.10** ± 0.00			M31031
RGIAL76	glutathione peroxidase I	0.88 ± 0.01	1.06 ± 0.23	**0.62** ± 0.00	X12367
Transport and binding proteins					
RNAB88	α-2u-globulin	**0.10** ± 0.00			AB039822
RGIAJ72	serum albumin	**0.55** ± 0.05			P02770
RGIBB41	β-globin	**0.40** ± 0.06	**0.23** ± 0.01	**0.45** ± 0.10	X16417
RGIAJ13	hemoglobin α-chain	**0.47** ± 0.02	**0.28** ± 0.03	**0.32** ± 0.07	NM_013096
RGIAA46	β_2-microglobulin	0.80 ± 0.09	**0.61** ± 0.04	0.85 ± 0.12	NM_012512
RGIAA50	ferritin heavy chain	0.88 ± 0.04	0.67 ± 0.06	**0.58** ± 0.08	NM_012848
RGIAA74	ferritin light chain subunit	**0.54** ± 0.04	1.22 ± 0.32	**0.54** ± 0.03	L01122
Unassigned					
RGIAA36	TSC-22 putative transcriptional regulator	1.07 ± 0.07	0.98 ± 0.11	**0.65** ± 0.02	L25785
RGIAC32	Tu transition elongation factor (human)	0.89 ± 0.04	**0.63** ± 0.03	0.67 ± 0.02	AU035161
RGIBC14	glycoprotein-assoc. amino acid transporter/BAT1			**0.26** ± 0.08	AW140996
RGIAQ94	Na^+/H^+ exchange protein isoform 1		**0.58** ± 0.07		M85299
RGIAZ37	peptidylarginine deiminase type IV (PDI4)		**0.32** ± 0.06		NM_017227
RGIAI18	endoplasmatic reticulum transmembrane protein	**0.55** ± 0.09	0.79 ± 0.01	0.86 ± 0.06	NM_011631
RGIAI87	cyclin A[a]		**0.51** ± 0.10		Z26580

Transcripts underexpressed in tissues from hypophysectomized compared to normal rats. List of genes with expression levels with an average decrease of more than 1.5-fold in mRNA isolated from liver, heart, or kidney, as measured by the Cy5/Cy3 ratio. Identity of columns, from *left* to *right*: TIGR rat gene index ID, gene name, average fold change observed in liver, range of the two independently measured ratios in liver, the same for heart and kidney, and GenBank accession number. The average fold change value is left blank for some genes in some tissues because no signal was detected (<1.6 times above background). Ratios smaller than 0.67 (down-regulated >1.5 times) are shown in *bold*.

[a] Genes that have not been sequence verified.

coneogenesis from alanine, but not lactate, is increased, as is the amino acid transport (27) and glutamic-pyruvate transaminase activity (35) and expression (Table 3). This increase in gluconeogenesis occurs despite a reduced expression of glucose-6-phosphatase and fructose-1,6-biphosphatase (Table 2). It may be the case that this reduced expression is sufficient for the requirements of the normally fed rat, but not for the fasted animal. It is well known that Hx animals are unable to maintain blood glucose levels during fasting. When fasting, the animals develop hypoglycemia despite a decrease in the glycogen storages in liver, muscle, and heart (3, 26). Seemingly, there is an uncoupling effect between glycogen degradation and glucose secretion. Glycogen degradation is regulated by the enzyme glycogen phosphorylase, which is posttranslationally activated through adenylate cyclase-induced phosphorylation. Increased usage of glycogen can be explained by the known hypersensitivity to glucagon observed in the liver of Hx animals (36). On the other hand, the secretion of glucose from liver requires its previous dephosphorylation by glucose-6-phosphatase, an enzyme whose expression (Table 2) and activity (37) are greatly reduced by hypophysectomy. The other source of blood glucose during fasting is the gluconeogenic pathway. The expression of the rate-limiting enzyme in this pathway, fructose-1,6-biphosphatase, was also down-regulated by hypophysectomy in liver and kidney (Table 2), presumably due to adrenal insufficiency (38). It has been shown that the activity and expression of this enzyme can be induced by treatment with glucocorticoids (3).

The general decrease in metabolic rate is also reflected by a decrease in the secretory function. In our study this was reflected by liver expression of the mRNA encoding the main secreted liver protein, serum albumin (39), which, together with some other genes involved in the protein secretory pathway, showed reduced expression after hypophysectomy (Table 2). For example, the expression of the genes for endoplasmic reticulum-resident chaperone proteins such as calreticulin, which is involved in protein folding of *de novo* synthesized glycosylated proteins (40), was reduced. The same was seen for the expression of ADP-ribosylation factor 4, which is involved in vesicular trafficking between the endoplasmic reticulum and the *cis*-Golgi compartment (41). Moreover, farnesyl pyrophosphate synthetase, a rate-limiting enzyme involved in the cholesterol biosynthetic pathway (42), and stearyl-CoA desaturase 1 (SCD1), the key enzyme in the synthesis of unsaturated fatty acids (43), were also down-regulated in liver. Cholesterol and unsaturated fatty acids are essential for maintaining membrane fluidity and are therefore subject to a coordinate regulation that is achieved through the activation of common transcription factors, sterol regulatory element-binding transcription factors (44). Response elements for these factors are also present in the promoters of 3-hydroxy-3-methylglutaryl-coenzyme A reductase and low density lipoprotein (LDL) receptor genes. These genes are also involved in lipid metabolism and are known to be down-regulated by hypophysectomy (45). These findings may reflect the known co-

TABLE 3. Effects of hypophysectomy, up-regulated genes

		Cy5/Cy3 ratio Hx/normal			Accession no.
		Liver	Heart	Kidney	
Energy metabolism					
RGIAA29	ATP synthase *c* subunit, P2 gene		**1.74** ± 0.07	1.26 ± 0.17	D13124
RGIAA07	cytochrome *c* oxidase subunit VIII		**1.65** ± 0.04	1.06 ± 0.12	L48209
RNAC75	brown fat uncoupling protein		**1.53** ± 0.03		NM_012682
Fatty acid metabolism					
RGIBB50	peroxisome proliferator-activated receptor α	**1.64** ± 0.18	1.05 ± 0.15	1.11 ± 0.18	NM_013196
RGIAA64	2,4-dienoyl-CoA reductase	**2.05** ± 0.16	**0.55** ± 0.10	0.98 ± 0.30	D00569
RNAB87	apolipoprotein E	0.97 ± 0.05	0.99 ± 0.07	**2.16** ± 0.53	S76779
RGIBC49	lipoprotein lipase		1.06 ± 0.34	**2.46** ± 0.52	NM_0.12598
Intermediary metabolism					
RGIAJ75	glutamate pyruvate transaminase	**1.63** ± 0.23	1.08 ± 0.12		D10354
RGIAW41	α-L-iduronidase (ldua)	**2.63** ± 1.32	**2.32** ± 0.43		NM_008325
RGIAC56	aromatic L-amino acid decarboxylase	1.05 ± 0.01	**2.26** ± 0.08	**0.63** ± 0.15	NM_012545
RNAC27	inosine 5′-monophosphate dehydrogenase (mouse)		**1.75** ± 0.16		NM_011830
Receptors/signal transduction					
RGIAQ27	glioma tumor suppressor candidate region gene 2	**2.11** ± 0.33	**1.84** ± 0.04	**1.99** ± 0.09	AW142592
RGIAR91	insulin-like growth factor-binding protein-2	**6.69** ± 2.46			M31672
RGIAN83	calponin, acidic isoform	**2.60** ± 0.32	1.20 ± 0.22	1.29 ± 0.19	U06755
RNAB53	calmodulin (RCM3)	**1.87** ± 0.24	1.22 ± 0.19	1.32 ± 0.33	M19312
RGIBB95	cAMP phosphodiesterase 4B	**2.12** ± 0.15	1.00 ± 0.12		AW141098
RGIAV91	integral membrane protein α-chain, MHC RT1-B1	1.47 ± 0.13	0.98 ± 0.06	**1.76** ± 0.20	X14879
RGIAC53	Rab GDP-dissociation inhibitor, α		**1.63** ± 0.02	1.42 ± 0.47	AF130987
RNAC57	brain natriuretic peptide		**1.85** ± 0.32		M25297
RGIAC25	tubulin, γ		**1.76** ± 0.20		AB015946
RNAC31	slow/cardiac troponin C isoform 3 (mouse)		**1.68** ± 0.11		NM_009393
RGIAW47	plasma membrane calcium ATPase isoform 1		**1.82** ± 0.03		L04739
Apoptosis					
RGIAF55	E1B 19K/Bcl-2-binding protein homolog	**2.08** ± 0.44	1.19 ± 0.09	1.24 ± 0.02	AF243515
RGIAF11	apoptosis inhibitor Bcl-x		**1.88** ± 0.02	1.33 ± 0.17	X82537
Cell structure					
RGIAJ60	bone proteoglycan II (decorin)		**0.66** ± 0.02	**1.68** ± 0.17	X59859
RNAA25	heart myosin light chain 2		**1.92** ± 0.02		X07314
Transcription/RNA processing/transcription factors					
RGIAG73	transcription elongation factor TFIIS.h		1.72 ± 0.41	1.47 ± 0.49	AJ223472
RGIAA57	La/SS-B protein	1.09 ± 0.16	**1.65** ± 0.07	1.14 ± 0.20	X67859
RGIAK08	transcription factor ear-2	**1.76** ± 0.09		**1.60** ± 0.01	L25674
RGIAQ63	DNA-binding protein inhibitor ID-1		1.26 ± 0.10	**3.47** ± 0.06	NM_0.12797
RGIAU76	inhibitor of DNA binding 3, ID-3	0.81 ± 0.01	1.07 ± 0.10	**2.42** ± 0.20	NM_013058
RGIAQ07	small nuclear ribonucleoprotein SmD2			**1.60** ± 0.01	U15008
Protein synthesis/stabilization and folding					
RGIAA49	ribosomal protein S19	**1.80** ± 0.31	**1.78** ± 0.12	**1.59** ± 0.27	X51707
RGIAC03	ribosomal protein L10	**1.67** ± 0.53	**1.84** ± 0.21	**1.63** ± 0.68	L25899
RGIAG27	ribosomal protein L44	**1.63** ± 0.36	**2.40** ± 0.47	**2.25** ± 0.94	P09896
RGIAA37	ribosomal protein L37a	1.41 ± 0.04	**1.84** ± 0.20	**1.99** ± 0.01	X14069
RGIAA61	ribosomal protein S9	1.37 ± 0.01	**1.79** ± 0.29	**1.53** ± 0.19	X66370
RGIAA89	ribosomal protein L32	1.22 ± 0.01	**1.87** ± 0.02	**2.06** ± 0.12	X06483
RGIAZ14	ribosomal protein S15a	1.12 ± 0.17	0.98 ± 0.07	**1.51** ± 0.01	X77953
RGIAP55	ribosomal protein L7	**2.06** ± 0.16	**1.65** ± 0.07	**1.81** ± 0.00	X57961
RGIAA67	ribosomal protein S4	**1.57** ± 0.04	**1.70** ± 0.07	**1.98** ± 0.17	M19393
RGIAG67	ribosomal protein S18	1.42 ± 0.09	**1.66** ± 0.21	**1.63** ± 0.06	X57529
RGIAG39	ribosomal protein L9	1.36 ± 0.01	**2.00** ± 0.46	**1.88** ± 0.05	X51706
RGIAE43	ribosomal protein L19	1.35 ± 0.11	**1.63** ± 0.00	**1.84** ± 0.31	J02650
RGIAG91	ribosomal protein S23	1.26 ± 0.07	**1.76** ± 0.31	**1.92** ± 0.07	X77398
RNAC42	ribosomal protein L5		1.06 ± 0.21	**1.88** ± 0.29	X06148
RNAC23	ribosomal protein S8	**1.83** ± 0.28	**1.60** ± 0.31	**1.56** ± 0.20	X73829
RGIAA71	ribosomal protein L34	1.17 ± 0.10	**1.91** ± 0.18	1.37 ± 0.14	X14401
RGIAA69	ribosomal protein L23a	**1.82** ± 0.20	**1.65** ± 0.14	**2.01** ± 0.13	X65228
RNAC49	laminin receptor 1 (Lamr1)/40-kDa ribosomal protein		**1.55** ± 0.01	**1.68** ± 0.56	NM_017138
Protein degradation					
RGIAB33	ubiquitin and ribosomal protein S27a fusion protein	**1.64** ± 0.42	**1.92** ± 0.21	**1.94** ± 0.64	X81839
Transport and binding proteins					
RGIAI10	carbonic anhydrase III (CA3)	0.78 ± 0.09		**3.38** ± 1.17	AF037072

ordination between the synthesis of secreted proteins and the biogenesis of the endoplasmic reticulum (46).

Many gene products involved in signal transduction were markedly down-regulated by hypophysectomy in a tissue-specific nature. For example, calbindin D was down-regulated in kidney, but it does not appear to be expressed in

TABLE 3. Continued

		Cy5/Cy3 ratio Hx/normal			Accession no.
		Liver	Heart	Kidney	
Unassigned					
RGIAI05	heat-stable antigen CD24		**1.74** ± 0.14		U49062
RNAD84	human desmoplakin I	**1.96** ± 0.02	0.94 ± 0.20		M77830
RGIAJ92	urinary protein 2 precursor/ATPase inhibitor	**1.68** ± 0.23		0.78 ± 0.14	AF198441
RGIAC09	lens epithelial protein	1.49 ± 0.31	**1.68** ± 0.15	**1.55** ± 0.47	AW914226
RGIAV75	deoxyribonuclease I			**1.64** ± 0.07	NM_013097
RGIAR37	mouse cyclin 1	1.36 ± 0.19	**1.71** ± 0.15		AF228740
Reference genes					
RGIAA01	β-actin	1.08 ± 0.24	1.23 ± 0.19	0.78 ± 0.20	V01217
RGIAA03	β-actin	1.09 ± 0.15	1.23 ± 0.15	0.81 ± 0.23	X03672
RGIAF54	histone H1	1.04 ± 0.08	1.01 ± 0.29	0.92 ± 0.06	387193

Transcripts overexpressed in tissues from hypophysectomized compared to normal rats. List of genes with expression levels with an average increase of more than 1.5-fold in mRNA isolated from liver, heart, and kidney, as measured by the Cy5/Cy3 ratio. Identity of columns, from *left* to *right*: TIGR rat gene index ID, gene name, average fold change observed in liver, range of the two independently measured ratios in liver, the same for heart and kidney, and GenBank accession number. The average fold change value is left blank for some genes in some tissues because no signal was detected (<1.6 times above background). Ratios greater than 1.5 are shown in *bold*.

heart or liver. The expression of this gene together with osteonectin and matrix Gla protein (MGP) is regulated by 1,25-dihydroxycholecalciferol, the active hormone derived from vitamin D (47, 48). 1,25-Dihydroxycholecalciferol synthesis by the renal 25-hydroxyvitamin D_3-1α-hydroxylase is known to be reduced by hypophysectomy in rats (49, 50). The low levels of this enzyme may explain why these genes are affected. Osteonectin and MGP are matrix proteins involved in the remodeling of the extracellular matrix and the regulation of bone formation. Mice that are deficient in osteonectin develop severe osteopenia (51). On the other hand, MGP knockout mice exhibit inappropriate calcification of various cartilages and arteries, which eventually lead to short stature and osteopenia (52). Besides many other effects, hypophysectomy causes profound osteopenia, primarily due to an inhibition of bone gain, and a decrease in bone turnover (53). It will therefore be interesting to analyze the expression of these genes in bone tissue and their involvement in bone turnover during hypophysectomy. These genes are also expressed in tissues others than bones. Osteonectin, for example, is also expressed by myocardocytes (54), liver myofibroblasts (55), and glomerular mesangial cells (56), where it regulates the synthesis and deposition of extracellular matrix proteins such as collagen.

In all of the tissues analyzed, hypophysectomy caused a marked reduction of genes expressing α-hemoglobin and β-globin. The lack of tissue specificity of this effect is expected, because the mRNA is most likely to come from circulating erythrocytes trapped within the tissues (nonperfused tissues were used). This may reflect the well known physiological effects. After hypophysectomy, rats became anemic with a decrease in hematocrit, hemoglobin concentration, red blood cell count, and reticulocytes (57). GH can restore normal levels of red blood cells independently of other hormones (58).

Approximately 100 different genes were found to be overexpressed (>1.5-fold) in mRNA from Hx animals compared with similar amounts of mRNA from normal animals in the three tissues analyzed. Of the 62 genes of known function found to be overexpressed in mRNA of Hx rat tissues, 18 were related to the protein synthesis machinery, including the protein components of the ribosome and translation factors. Ribosomal proteins are stochiometrically assembled with ribosomal RNA to form the ribosomal subunits. Consequently, the expression of these genes is coregulated, and the existence of such a cluster of coregulated ribosomal genes was shown here as well as in previous studies using yeast and mammalian cell cultures (59). The genes belonging to this cluster were approximately 1.4–2 times overexpressed in the mRNA population from Hx compared with normal tissue. This result is in line with previous studies regarding the effect of hypophysectomy on protein synthesis. The severe inhibition of protein synthesis after removal of the pituitary gland is mainly due to a general reduction in mRNA content rather than a diminished number of ribosomes (23, 60, 61). The diminished protein synthesis rate by isolated hepatic ribosomes from Hx rats can be restored by adding exogenous RNA to these preparations (62).

In contrast to underexpressed genes, fewer genes were overexpressed across all three tissues of Hx rats beyond the levels of those belonging to the ribosomal cluster. As an example, ID-1, ID-3, and carbonic anhydrase III were found to be overexpressed in Hx kidney, but not in heart or liver. ID (inhibitors of DNA binding) proteins are helix-loop-helix proteins that form heterodimers with ubiquitous and/or tissue-specific basic helix-loop-helix proteins, and thereby inhibit their DNA-binding activity. The interaction between ID-1 or ID-3 and basic helix-loop-helix proteins, many of which are essential for cellular differentiation, has been proposed as a key regulatory event leading to negative regulation of cell differentiation and as positive regulators of G_1 cell cycle control (63, 64). Carbonic anhydrase, a key enzyme in the regulation of acid-base balance by the kidney is involved in acidification of the urine and reabsorption of HCO_3^- (65). It may be the case that low food intake and diminished erythropoiesis (66) and albumin production (39) after hypophysectomy result in metabolic acidosis. Thus, a compensatory mechanism in kidney may be triggered by the induction of the expression of carbonic anhydrase (67).

In liver, the type IV cAMP-specific phosphodiesterase-4B was overexpressed beyond the levels of the ribosomal cluster. The type IV isoforms of phosphodiesterases are ex-

pressed in liver and regulated by glucagon at the level of enzymatic activity (68) and by cAMP at the level of gene expression (69). This finding may be related to the report of an increase in both basal and glucagon-stimulated adenylate cyclase activity in hepatocytes from Hx rats (70). An increased cAMP production may activate glycogen phosphorylase and account for the decrease in glycogen content found in Hx animals after fasting (3, 27).

Effects of chronic GH treatment on gene expression

Chronic hGH treatment of Hx animals results in clear restoration of body growth among other physiological changes, which in our view justify the selection of this mode of treatment for the study. The discussion below has been focused on relating the variations seen in gene expression to those physiological changes. No attempts have been made to distinguish between the lactogenic and the somatogenic effects of human GH in nonhepatic tissues, as such a study would require a different experimental design. In the interpretation of our results it is important to stress that the state of hypophysectomy creates a physiological situation unique to the model itself (of multiple hormonal deficiency), and this should be kept in mind when interpreting the effects of GH.

As already mentioned, the primary characteristic of the Hx animal is growth failure consequent to the loss of GH. Accompanying this are multiple alterations in the intermediary metabolism. Thus, the Hx animal has a higher proportion of fat and a lower proportion of protein in its carcass. Chronic hGH administration restores the growth rate, improves the nitrogen balance, and reverses the changes in body composition found in Hx animals (3, 30). The protein anabolic effects of GH include increased uptake of amino acids in peripheral tissues, decreased protein breakdown and usage of amino acids for energy expenditure, and increased protein synthesis (71, 72). These effects are partly accomplished by increasing the utilization of FFA in peripheral tissues. Thus, GH treatment promotes lipolysis and prevents lipogenesis in adipose tissue, which increases the availability of FFA for energy expenditure (4, 33). The liver plays a key role in the regulation of lipid metabolism by its production and uptake of lipoproteins. GH affects hepatic lipid metabolism on many levels, including increased lipoprotein and triglyceride production, increased secretion of very low density lipoprotein, and increased expression of LDL receptors (73).

In the present study the strong effects of hypophysectomy on the expression of genes involved in the metabolism of carbohydrates and lipids and in ATP production reflect the importance of pituitary hormones in cellular homeostasis. Furthermore, the modest number of genes that showed normalized expression levels in Hx rats upon chronic hGH administration substantiates the importance of other pituitary hormones in the control of metabolic pathways. The loss of TSH and ACTH leads to secondary thyroid and adrenal insufficiency, whereas FSH and LH depletion lead to a reduced production of sex steroids. The gene encoding glucose-6-phosphatase, the final enzyme in the gluconeogenic pathway, which showed a decreased expression in liver and kidney from our Hx rats, could exemplify this. The activity of this enzyme is known to be induced by GH in Hx rats substituted with thyroid hormone and ACTH (37). The GH-mediated effect on this gene product was recently shown to include induced mRNA expression in the liver of aged rats, which are not as deficient in thyroid and adrenal hormones as the Hx rats (our unpublished results). Similarly, hepatic glutamate dehydrogenase mRNA expression is induced by GH in aged rats (our unpublished results), but only showed decreased expression in our Hx rats and no major changes in mRNA levels after GH administration. In contrast, the hepatic expression of glutamate pyruvate transaminase was induced in Hx rats compared with normal rats and was repressed upon hGH treatment. This finding confirms results obtained measuring the enzymatic activity and is in line with previous reports on GH-mediated down-regulation of urea synthesis and increased nitrogen balance (74).

Other genes may also contribute to the anabolic effects of GH. For example, transketolase expression (together with other enzymes in the pentose phosphate pathway) was reduced after hypophysectomy and was induced after GH treatment (Table 4). This enzyme participates in the synthesis of ribose phosphate, which is needed for the production of RNA. In line with this is the finding that the expression of xanthine dehydrogenase, which is responsible for the degradation of nucleotides, was down-regulated in liver by GH. A reduced level of mRNA expression was observed for peroxisome proliferator-activated receptor α (PPARα) in GH-treated Hx rats together with gene products previously shown to be regulated by PPARα, including 2,4-dienoyl-CoA reductase and 3-hydroxyacyl-CoA dehydrogenase (75, 76). The increased expression of PPARα observed in Hx rats might therefore be a consequence of GH deficiency.

Analysis of GH-induced changes in gene expression showed that most of the effects were highly tissue specific. Liver was the most responsive tissue, which agrees with previous findings about GH receptor expression and GH sensitivity of this tissue (77). The expression of SCD1 was greatly reduced in liver after hypophysectomy and was induced by GH treatment (Fig. 2). SCD catalyzes the oxidation of stearyl CoA to form the monounsaturated fatty acid oleyl-CoA. Oleic acid is the major unsaturated fatty acid in lipid stores, fat tissue, and membrane phospholipids. The ratio of stearic/oleic acid has been implicated in the regulation of cell growth and differentiation through effects in cell membrane fluidity and signal transduction. An increased ratio of stearic to oleic acid, for example, induces apoptosis in rat ventricular myocytes, whereas oleate-enriched LDL is resistant to oxidation (78, 79). Enzymes involved in detoxification and xenobiotic metabolism were also GH regulated in liver (Table 4), which is in agreement with previous reports (6).

In heart, GH induced the expression of atrial natriuretic peptide in Hx rats. These animals otherwise show low levels of this factor and attenuated diuresis and natriuresis in response to blood volume expansion (80). The expression levels of the hemoglobin genes were also restored, a finding in line with the known positive actions of GH on hemopoiesis (58). The hormonal regulation of vasculogenesis deserves more attention, keeping in mind the known positive effects of GH (81). The present study shows that GH down-regulates the liver expression of collagen type XVIII, the precursor of endostatin, a newly described inhibitor of angiogenesis. In

TABLE 4. GH treatment in Hx rat

		Ratio Hx + GH/Hx			Accession no.
		Liver	Heart	Kidney	
Carbohydrate and energy metabolism					
RGIAX15	transketolase	**1.80** ± 0.17	1.41 ± 0.12	**0.65** ± 0.07	AW140979
RGIAX71	cytochrome *b*5, mitochondrial isoform	**1.77** ± 0.00	1.27 ± 0.03	1.03 ± 0.01	Y12517
RGIAR15	ubiquinol-cytochrome *c* reductase hinge protein	**1.72** ± 0.06	1.36 ± 0.23	0.70 ± 0.05	AW142278
RGIAA84	aldehyde reductase	0.71 ± 0.01	**0.66** ± 0.05	0.85 ± 0.11	D10854
RGIAA10	aldose reductase		**0.66** ± 0.00	0.97 ± 0.04	X05884
RGIAK80	glucose transporter type 1 (GUT1)		**0.64** ± 0.08	1.29 ± 0.13	M13979
Fatty acid/cholesterol metabolism					
RGIAE62	stearyl-CoA desaturase	**2.65** ± 0.18			J02585
RGIBC21	acyl-CoA synthetase 5	**1.74** ± 0.09			AB012933
RGIAV01	fatty acid transporter	**2.09** ± 0.26	0.98 ± 0.03	0.70 ± 0.14	AB005743
RGIAE78	3-hydroxyacyl-CoA dehydrogenase	**0.67** ± 0.01	0.69 ± 0.09	1.05 ± 0.12	AF095449
RGIBB50	peroxisome proliferator-activated receptor-α	**0.58** ± 0.05	0.81 ± 0.09	1.09 ± 0.14	NM_013196
RGIBC49	lipoprotein lipase		1.12 ± 0.02	**0.42** ± 0.06	NM_012598
RGIAA64	2,4-dienoyl-CoA reductase	**0.61** ± 0.02	0.99 ± 0.14	1.01 ± 0.13	D00569
RNAB87	apolipoprotein E	1.10 ± 0.07	**1.58** ± 0.20	0.65 ± 0.09	S76779
Intermediary metabolism					
RGIAO17	glutamate dehydrogenase	0.83 ± 0.01	**0.64** ± 0.00	0.91 ± 0.15	X14044
RGIAJ75	glutamate pyruvate transaminase	**0.56** ± 0.04		1.09 ± 0.05	D10354
RGIAF36	xanthine dehydrogenase	**0.55** ± 0.04	0.76 ± 0.04	0.96 ± 0.02	NM_017154
Signal transduction/trophic factors					
RGIAO22	hepatic fibrinogen/angiopoietin-related protein (HARP)	**1.61** ± 0.03			AF169313
RNAA89	fibrinogen γ-chain	**1.90** ± 0.00			J00734
RGIAJ94	atrial natriuretic peptide		**1.78** ± 0.27		NM_012612
RGIBB30	stanniocalcin I		0.81 ± 0.13	**1.80** ± 0.13	NM_003155
RGIAB63	14-3-3 protein, ζ-subtype	**1.68** ± 0.11	1.37 ± 0.22	0.82 ± 0.17	U37252
RGIAJ86	angiotensinogen	**0.62** ± 0.03			L00091
RGIBA44	calbindin D28			**0.45** ± 0.06	M27839
Cell structure					
RGIAM23	collagen α1 type III	**2.11** ± 0.38	**2.22** ± 0.40	1.15 ± 0.14	X70369
RGIAA83	Amyloid precursor-like protein (mouse)	**1.87** ± 0.08		1.17 ± 0.13	NM_007467
RGIBC35	myosin heavy chain (MHC)		**1.58** ± 0.02		AW141106
RGIAQ36	fibronectin	0.72 ± 0.01	0.95 ± 0.10	**1.88** ± 0.35	X15906
RGIBA52	collagen α1 type XVIII/endostatin precursor	**0.61** ± 0.04		1.34 ± 0.04	AJ236873
RGIAE02	tubulin, α-2	0.78 ± 0.09	**0.66** ± 0.01	1.08 ± 0.22	M13446.1
Transcription/RNA processing					
RGIAQ63	DNA-binding protein inhibitor ID-1		**1.60** ± 0.10	1.00 ± 0.09	NM_012797
RGIBC61	transcription factor TFIID, subunit TAFII28		**1.68** ± 0.02		AW144742
RGIAA36	TSC-22 putative transcriptional regulator	0.67 ± 0.04	**0.65** ± 0.00	1.00 ± 0.05	L25785.1
RGIAK08	transcription factor ear-2[a]	**0.62** ± 0.02		1.26 ± 0.02	L25674
Protein degradation					
RGIAV42	meprin A β-subunit			**1.50** ± 0.12	NM_013183
Detoxification/xenobiotic metabolism					
RGIAJ41	UDP-glucose dehydrogenase	1.46 ± 0.02		**0.61** ± 0.07	AB013732
RNAA87	gluthatione-*S*-transferase class π (bovine)	**2.99** ± 0.14			X61233
RNAB61	PRx III (peroxiredoxin)	**2.14** ± 0.10	1.33 ± 0.23	0.85 ± 0.06	AF106944
RNAA80	CYP2C12	**2.00** ± 0.15			M33656
RGIAJ76	CYP2C7	**4.11** ± 0.98			M31031
RGIAE92	CYP2A2 (steroid hydroxylase IIA2)	**0.65** ± 0.02	0.96 ± 0.18	1.25 ± 0.11	M33313
RNAA94	CYP3A2	**0.47** ± 0.03			U09742
Transport and binding proteins					
RGIAI73	β-globin	**1.52** ± 0.16	**1.55** ± 0.46	1.22 ± 0.21	X16417
RGIAJ13	hemoglobin α-chain	1.44 ± 0.05	**2.39** ± 0.99	1.31 ± 0.00	NM_013096
RGIAI10	carbonic anhydrase III (CA3)	**0.42** ± 0.10		**0.27** ± 0.09	AF037072
RGIAA50	ferritin heavy chain	**0.62** ± 0.03	0.90 ± 0.14	1.24 ± 0.20	NM_012848
RNAA71	transferrin (Tf)	**1.71** ± 0.13		1.00 ± 0.26	NM_017055
Unassigned					
RGIBC14	glycoprotein-associated amino acid transporter/BAT1			**2.26** ± 0.09	AW140996
RGIAD72	sulfated glycoprotein 2	**0.57** ± 0.04	1.19 ± 0.10	1.06 ± 0.07	X13231
RGIAG76	N-myc downstream-regulated 2 (Ndr2)	**0.65** ± 0.00	0.80 ± 0.11	0.92 ± 0.14	NM_013864
RNAA21	complement component 3 (C3)	**1.77** ± 0.12			NM_016994
Reference genes					
RGIAA01	β-actin	0.89 ± 0.05	1.07 ± 0.00	1.06 ± 0.19	V01217
RGIAA03	β-actin	0.89 ± 0.06	1.05 ± 0.06	1.07 ± 0.22	X03672
RGIAF54	histone H1	**0.64** ± 0.04	**0.64** ± 0.09	1.28 ± 0.17	J03482

Transcripts regulated by GH in the hypophysectomized rat. List of genes that increase or decrease an average of more than 1.5-fold in GH-treated liver, heart, and kidney from hypophysectomized rats as measured by Cy5/Cy3 ratio. Identity of columns, from *left* to *right*: TIGR rat gene index ID, gene name, average fold change observed in liver, range of the two independently measured ratios in liver, the same for heart and kidney, and GenBank accession number. The average fold change value is left blank for some genes in some tissues because no signal was detected (<1.6 times above background). Ratios greater than 1.5 or smaller than 0.67 are shown in *bold*.

[a] Genes that have not been sequence verified.

the same tissue hypophysectomy reduces and GH induces the expression of the hepatic fibrinogen/angiopoietin-related protein, a recently described secreted protein that prevents endothelial cell apoptosis (82). These findings may constitute a novel mechanism used by GH to regulate the cardiovascular system.

In kidney, GH stimulated the expression of stanniocalcin I, a newly characterized hormone in mammals that stimulates phosphate reabsorption at proximal tubules (83). This finding may explain previous observations describing a positive effect of GH on phosphate transport in Hx rats fed a low phosphorous diet as well as in GH-deficient children (84). Regulation of stanniocalcin I by GH may be an important mechanism for positive calcium balance and the induction of longitudinal bone growth that is unique to GH action (85, 86). Several genes involved in kidney damage, such as fibronectin, collagen, and meprin A, were induced by GH in kidney (87, 88). Meprin A is the major matrix-degrading metalloproteinase in rat kidney. Its expression is polarized, as it is expressed in the brush border membrane of renal proximal tubular epithelial cells (89). It has been suggested that meprin A plays a role in the pathophysiology of acute renal failure after a variety of injuries to the kidney. Redistribution of this metalloendopeptidase to the basolateral membrane domain during acute renal failure results in degradation of the extracellular matrix and damage to adjacent peritubular structures (89, 90). It is a well known phenomenon that chronic GH treatment may result in kidney damage, and that hypophysectomy protects against glomerulosclerosis induced by diabetes (9). It will be interesting to further explore the role of GH-regulated genes such as meprin A in the development of diabetic nephropathy as well as the role of GH in this process.

The cDNA array used here contains a small sampling of rat cDNA transcripts and is far from a complete representation of all rat cDNAs. The present study therefore only represents a partial survey of genes regulated by hypophysectomy or genes responsive to GH treatment. Furthermore, the experimental models used, *i.e.* hypophysectomy and chronic hGH infusion, provide only partial information. The effects of hypophysectomy are dependent on developmental stage, and the effects of GH are dose dependent, significantly depend on the mode of administration (6), and if the GH treatment is long term would include actions of induced IGF-I. In extended studies the use of our cDNA microarray to study acute effects of GH on Hx animals would provide further insights into the tissue-specific changes, as the present study focused on a more stabilized condition after weeks of treatment. A study of the acute effects of GH on liver gene expression in another model of GH deficiency using a filter array of 588 rat cDNAs has recently been published (91). However, direct comparison to the present study cannot readily be made because a different experimental design was employed, and the two studies used a nonoverlapping set of GH-regulated genes.

In the present study cDNA arrays were used to characterize the state of hypophysectomy. Our data support earlier observations and can be put in a context of previous physiological knowledge. We also identified several genes that were previously not known to be regulated by either Hx or hGH treatment. In addition, a number of genes of unknown function (expressed sequence tags) responded to hypophysectomy and hGH in this study. In future experiments the value of DNA arrays to annotate such genes in terms of regulation will be studied. Further work is therefore required to extend our knowledge, and DNA array technology will serve as a valuable tool.

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