Growth Hormone Dependence of Somatomedin-C/Insulin-Like Growth Factor-I and Insulin-Like Growth Factor-II Messenger Ribonucleic Acids*

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The GH dependence of somatomedin-C/insulin-like growth factor I (Sm-C/IGF-I) and insulin like growth factor II (IGF-II) mRNAs was investigated by Northern blot hybridizations of polyadenylated RNAs from liver, pancreas, and brain of normal rats, untreated hypophysectomized rats, and hypophysectomized rats 4 h or 8 h after an ip injection of human GH (hGH). Using a ^{32}P-labeled human Sm-C/IGF-I cDNA as probe, four Sm-C/IGF-I mRNAs of 7.5, 4.7, 1.7, and 1.2 kilobases (kb) were detected in rat liver and pancreas but were not detectable in brain. In both liver and pancreas, the abundance of these Sm-C/IGF-I mRNAs was 8- to 10-fold lower in hypophysectomized rats than in normal rats. Within 4 h after injection of hGH into hypophysectomized animals, the abundance of liver and pancreatic Sm-C/IGF-I mRNAs was restored to normal. A human IGF-II cDNA was used as a probe for rat IGF-II mRNAs which were found to be very low in abundance in rat liver and showed no evidence of regulation by GH status. In pancreas, IGF-II mRNA abundance was below the detection limit of the hybridization procedures. The brain contained two IGF-II mRNAs of 4.7 and 3.9 kb that were 5-fold lower in abundance in hypophysectomized rats than in normal rats. These brain IGF-II mRNAs were not, however, restored to normal abundance at 4 or 8 h after ip hGH injection into hypophysectomized animals. To investigate further, the effect of GH status on abundance of Sm-C/IGF-I and IGF-II mRNAs in rat brain, a second experiment was performed that differed from the first in that hypophysectomized rats were given an injection of hGH into the lateral ventricle (intracerebroventricular injection) and a rat Sm-C/IGF-I genomic probe was used to analyze Sm-C/IGF-I mRNAs. In this experiment, a 7.5 kb Sm-C/IGF-I mRNA was detected in brain polyadenylated RNAs. The abundance of the 7.5 kb mRNA was 4-fold lower in hypophysectomized rats than in normal rats and was increased to 80% of normal within 4 h after icv administration of hGH to hypophysectomized animals. As in the first experiment, the abundance of the 4.7 and 3.9 kb brain IGF-II mRNAs was lower than normal in hypophysectomized rats. Brain IGF-II mRNAs were increased to 50% of normal in hypophysectomized rats given an icv injection of hGH but within 8 h after the injection rather than at 4 h as with Sm-C/IGF-I mRNAs. (Molecular Endocrinology 1: 233–242, 1987)

0888-8809/87/0233–0242$02.00/0
Molecular Endocrinology

INTRODUCTION

Somatomedin-C/ insulin-like growth factor I (Sm-C/IGF-I) is a mitogenic peptide that is thought to mediate many of the growth-promoting actions of GH (1, 2). Previous studies have established that serum and tissue concentrations of Sm-C/IGF-I are regulated by GH. In man, serum concentrations of Sm-C/IGF-I are higher than normal in acromegaly (3) and lower than normal in hypopituitary individuals (1). In the rat, concentrations of immunoreactive Sm-C/IGF-I in a number of tissues are reduced by hypophysectomy and restored to normal levels by GH replacement (4). Perfused rat liver also shows GH regulation of Sm-C/IGF-I biosynthesis and secretion (5). Serum and tissue concentrations of another somatomedin, insulin-like growth factor II (IGF-II), show a lesser degree of regulation by GH. In man, serum IGF-II levels are not elevated in acromegaly although some hypopituitary individuals do have lower serum IGF-II concentrations than normals (6). In contrast to Sm-C/IGF-I, the secretion of IGF-II by cultured

rat fibroblasts shows no evidence of regulation by GH (7).

In the present study we have investigated the effects of hypophysectomy and GH replacement on Sm-C/IGF-I and IGF-II mRNAs in rat liver, pancreas, and brain to establish whether previously observed effects of GH status on serum and tissue Sm-C/IGF-I concentrations are mediated at the level of mRNA abundance and whether GH status influences IGF-II mRNA abundance.

RESULTS

Effects of hypophysectomy and ip human GH (hGH) injection on Sm/IGF mRNA abundance

Four hybridizing Sm-C/IGF-I mRNAs with estimated sizes of 7.5, 4.7, 1.7, and 1.2 kilobases (kb) were observed in liver polyadenylated (poly A^+) RNAs (Fig. 1A). Signal intensities of all four hybridizing Sm-C/IGF-I mRNAs were lower in liver poly A^+ RNAs from hypophysectomized animals than in normals. Liver poly A^+ RnAs from hypophysectomized animals 4 or 8 h after hGH injection showed increased intensities of hybridizing Sm-C/IGF-I mRNAs compared with untreated hypophysectomized animals. Densitometric scanning of the 7.5 kb mRNA was performed to assess the relative abundance of the individual Sm-C/IGF-I mRNAs. Relative abundance of total hybridizing Sm-C/IGF-I mRNA was estimated also by densitometric scanning of dot blots of the liver poly A^+ RNAs. Both methods of densitometric analyses gave similar results (Fig. 2). The abundance of liver Sm-C/IGF-I mRNA was 8- to 10-fold lower in hypophysectomized rats compared with normal rats ($P < 0.01$), was higher in hypophysectomized animals 4 or 8 h after hGH injection than in untreated hypophysectomized animals ($P < 0.01$), and was not significantly different from normal in hypophysectomized animals 4 h after hGH injection ($P < 0.01$).

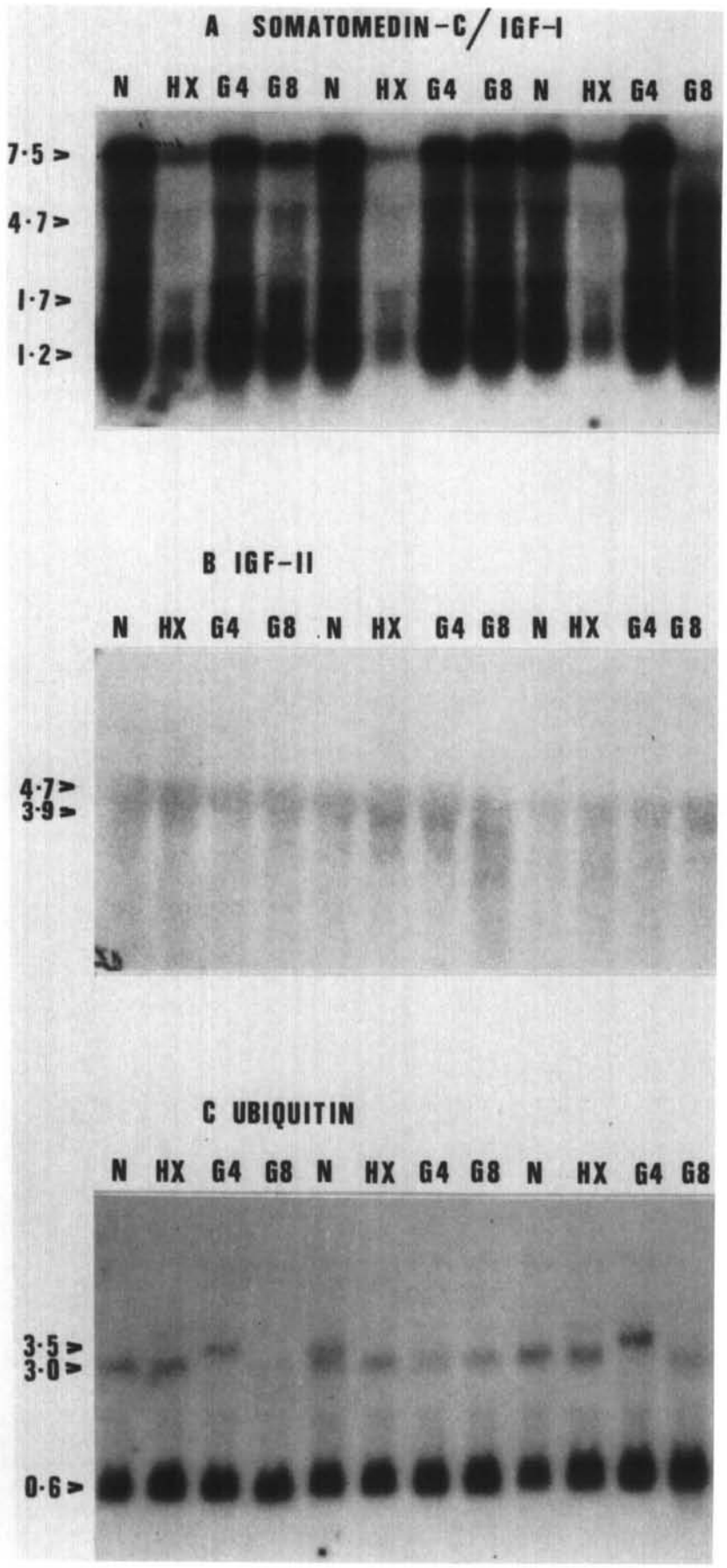
A replicate blot of liver poly A^+ RNAs probed with the human IGF-II cDNA probe, revealed minor amounts of 4.7 and 3.9 kb IGF-II mRNAs (Fig. 1B). Densitometric scanning of these IGF-II mRNAs revealed no significant changes with GH status (Fig. 2). As a control for possible differences in the amounts of liver poly A^+ RNAs applied to Northern blots, the blot shown in Fig. 1A was reprobed with a ^{32}P-labeled human ubiquitin cDNA (Fig. 1C). The abundance of a 0.6 kb ubiquitin mRNA, estimated by densitometric scanning, did not differ significantly in poly A^+ RNAs from the different groups of animals (Fig. 2).

As with liver, pancreatic poly A^+ RNAs contained four hybridizing Sm-C/IGF-I mRNAs although the abundance was lower in pancreas than liver (Fig. 3). The intensity of the 7.5 kb Sm-C/IGF-I mRNA, was used as a measure of the effect of GH status on the abundance of pancreatic Sm-C/IGF-I mRNAs. The abundance was 5- to 8-fold lower than normal in untreated hypophysectomized rats ($P < 0.05$), was higher in hypophysectomized rats treated with hGH for 4 h or 8 h than in the untreated hypophysectomized controls ($P < 0.05$), and was not significantly different from normal in hypophysectomized rats treated with hGH (Figs. 3 and 4). In pancreas, in contrast to the liver, the abundance of the 0.6 kb ubiquitin mRNA did vary with GH status. The changes in abundance of the 0.6 kb ubiquitin mRNA did not, however, parallel those of Sm-C/IGF-I mRNAs as this ubiquitin mRNA was higher in abundance in hypophysectomized rats than normal rats and showed more dramatic increases in abundance with hGH administration than did Sm-C/IGF-I mRNAs. Furthermore, densitometric scanning of a 1.5 kb ubiquitin mRNA, that was evident in pancreatic poly A^+ RNAs on long exposures of autoradiograms, revealed no significant changes in abundance with GH status (Fig. 4). These findings with pancreatic ubiquitin mRNAs, served to validate the observed GH dependent changes in pancreatic Sm-C/IGF-I mRNAs. IGF-II mRNAs were not detectable in pancreatic poly A^+ RNAs by the hybridization procedures employed (data not shown).

Sm-C/IGF-I mRNAs were not detectable in brain poly A^+ RNAs when the human Sm-C/IGF-I cDNA was used as probe (data not shown). Brain poly A^+ RNAs did, however, contain two mRNAs of 4.7 and 3.9 kb that hybridized with the human IGF-II probe. The signal intensity of these brain IGF-II mRNAs was lower than normal in untreated hypophysectomized and hGH-treated hypophysectomized rats (Fig. 5). The abundance of the brain IGF-II mRNAs was estimated by densitometric scanning (Fig. 6) and was found to be 5-fold lower in untreated hypophysectomized rats than in normal rats ($P < 0.01$). The abundance of brain IGF-II mRNAs also was lower than normal in hypophysectomized rats given an ip injection of hGH 4 h or 8 h before killing ($P < 0.01$). While the hypophysectomized animals given ip hGH at 8 h before killing showed some evidence for higher abundance of brain IGF-II mRNAs than untreated hypophysectomized animals (Figs. 5 and 6), the abundance was not significantly different in untreated and hGH-treated animals. The abundance of a 0.6 kb ubiquitin mRNA in brain poly A^+ RNAs did not differ significantly among the groups of animals (Fig. 6).

Fig. 1. GH Dependence of Liver Sm-C/IGF-I mRNAs

Autoradiograms of Northern blots of poly A^+ RNAs extracted from livers of normal (N), hypophysectomized (HX), and hypophysectomized rats 4 h (G4) or 8 h (G8) after ip injection of hGH. Fifteen microgram of each poly A^+ RNA were analyzed. Numbers at the left of the blots are the estimated sizes of hybridizing mRNAs based on comparison with denatured *Hin*dIII fragments of λ DNA electrophoresed on the same gels as the RNAs. A, Blot was hybridized with human Sm-C/IGF-I cDNA probe and exposed to x-ray film for 48 h. B, A replicate of the blot shown in A was hybridized with a human IGF-II cDNA probe and exposed to x-ray film for 6 days. C, The blot shown in A was stripped of hybridized Sm-C/IGF-I cDNA probe, rehybridized with a human ubiquitin cDNA probe, and exposed to x-ray film for 24 h. Note that polymorphisms in ubiquitin mRNAs and genes in different animals has been reported for *Xenopus* (24). The presence of 3 kb and/or 3.5 kb ubiquitin mRNAs (in addition to the major 0.6 kb ubiquitin mRNA) in the liver poly A^+ RNAs (and RNAs from other tissues shown in Figs, 3, 5, and 7) probably represents similar polymorphism in rat.



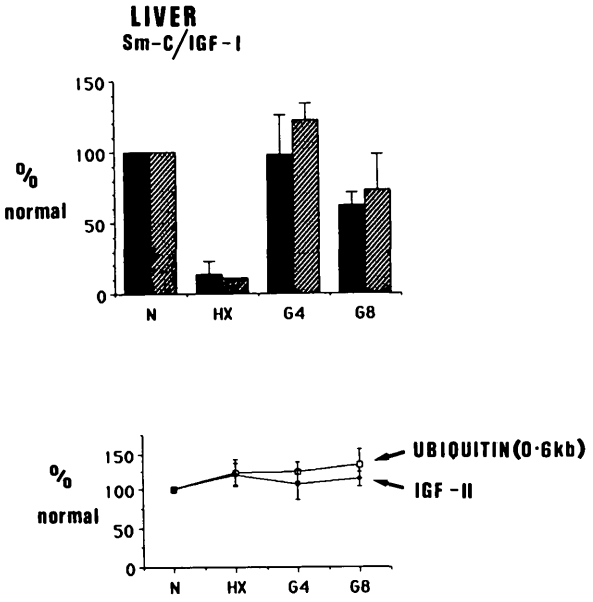


Fig. 2. Abundance of Liver Sm-C/IGF-I mRNAs
The histograms show the abundance Sm-C/IGF-I mRNA in poly A^+ RNAs from livers of normal (N), hypophysectomized (HX), and hypophysectomized rats 4 h (G4) or 8 h (G8) after ip injection of hGH. Estimates of Sm-C/IGF-I mRNA abundance represented by the ■ were obtained by densitometric scanning of the 7.5 kb Sm-C/IGF-I mRNA on autoradiograms of Northern blots. Those represented by ▨ were obtained from densitometric scanning of dot blots of the liver mRNAs. Abundance of liver IGF-II and 0.6 kb ubiquitin mRNAs, estimated by densitometric scanning of autoradiograms of Northern blots, are graphed in the lower panel for comparison. Abundance in each case is expressed as percentage of signal intensity observed in poly A^+ RNAs from normal liver. Each estimate of mRNA abundance shown is the mean ± SD of five different poly A^+ RNA preparations from five different animals. Statistical comparisons of mRNA abundance are described in the text.

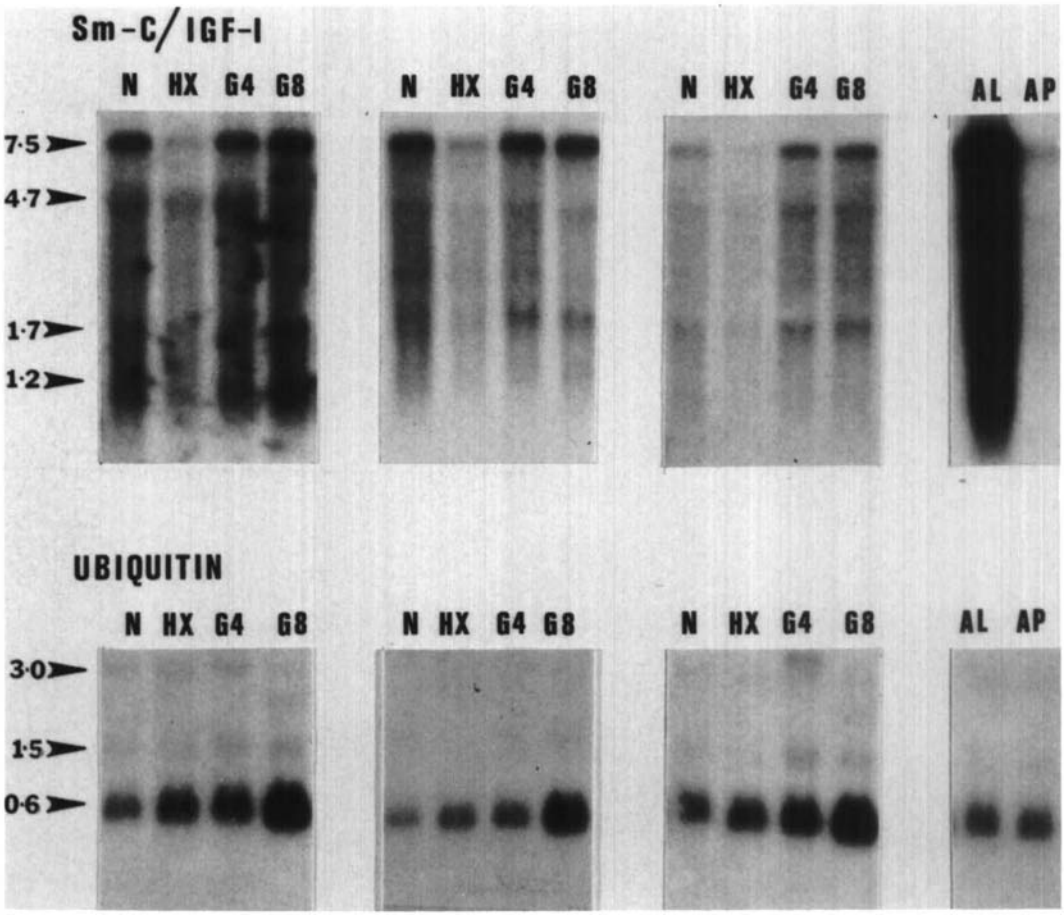
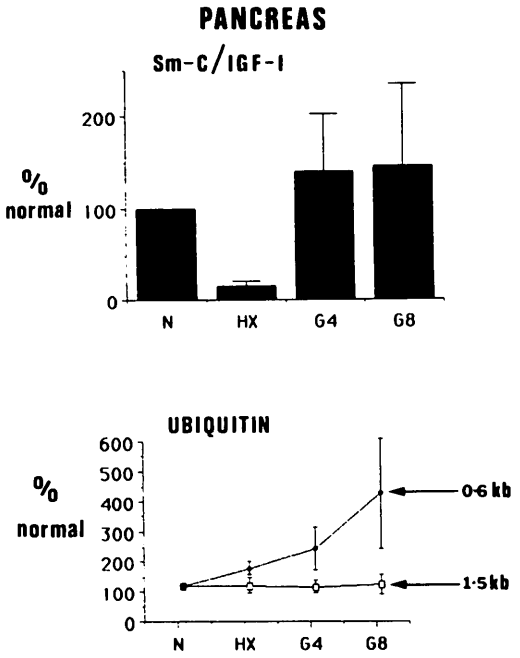


Fig. 3. GH Dependence of Pancreatic Sm-C/IGF-I mRNAs
Autoradiograms of Northern blots of 20 μg each of poly A^+ RNAs extracted from pancreas of normal (N), hypophysectomized (HX), and hypophysectomized rats 4 h (G4) or 8 h (G8) after ip injection of hGH. To illustrate that the abundance of Sm-C/IGF-I mRNAs in pancreas is much lower than that in liver, the blot at the far right shows a direct comparison of hybridization signals with 20 μg each of poly A^+ RNA from normal adult liver (AL) and normal adult pancreas (AP). *Numbers at the left* of the blots are the estimated sizes of hybridizing mRNAs based on comparison with denatured *Hin*dIII fragments of λ DNA electrophoresed on the same gels as the RNAs. Top, Blots were hybridized with human Sm-C/IGF-I cDNA probe and exposed to x-ray film for 72 h. (Blot of AL and AP RNAs was exposed for only 24 h.) Bottom, Blots shown at the top were stripped of hybridized Sm-C/IGF-I cDNA probe, rehybridized with the ubiquitin cDNA probe, and exposed to x-ray film for 24 h.



Effects of hypophysectomy and intracerebroventricular (icv) injection of hGH on brain Sm/IGF mRNAs

To further investigate the effects of hypophysectomy and hGH administration on brain Sm/IGF mRNAs, a second series of experiments was performed where hGH was injected directly into the lateral ventricle. In this series, a rat Sm-C/IGF-I genomic DNA probe was used to analyze brain Sm-C/IGF-I mRNAs with the

Fig. 4. Abundance of Pancreatic Sm-C/IGF-I mRNAs
The histogram at the top shows the abundance of the 7.5 kb Sm-C/IGF-I mRNA in poly A^+ RNAs from pancreas of normal (N), hypophysectomized (HX), and hypophysectomized rats 4 h (G4) or 8 h (G8) after ip injection of hGH. Abundance of 0.6 kb and 1.5 kb ubiquitin mRNAs, are graphed at the bottom for comparison. Abundance in each case is expressed as percentage of signal intensity observed in poly A^+ RNAs from normal pancreas. Each estimate of mRNA abundance shown is the mean ± SD of three different poly A^+ RNA preparations from three different animals. Statistical comparisons of mRNA abundance are described in the text.

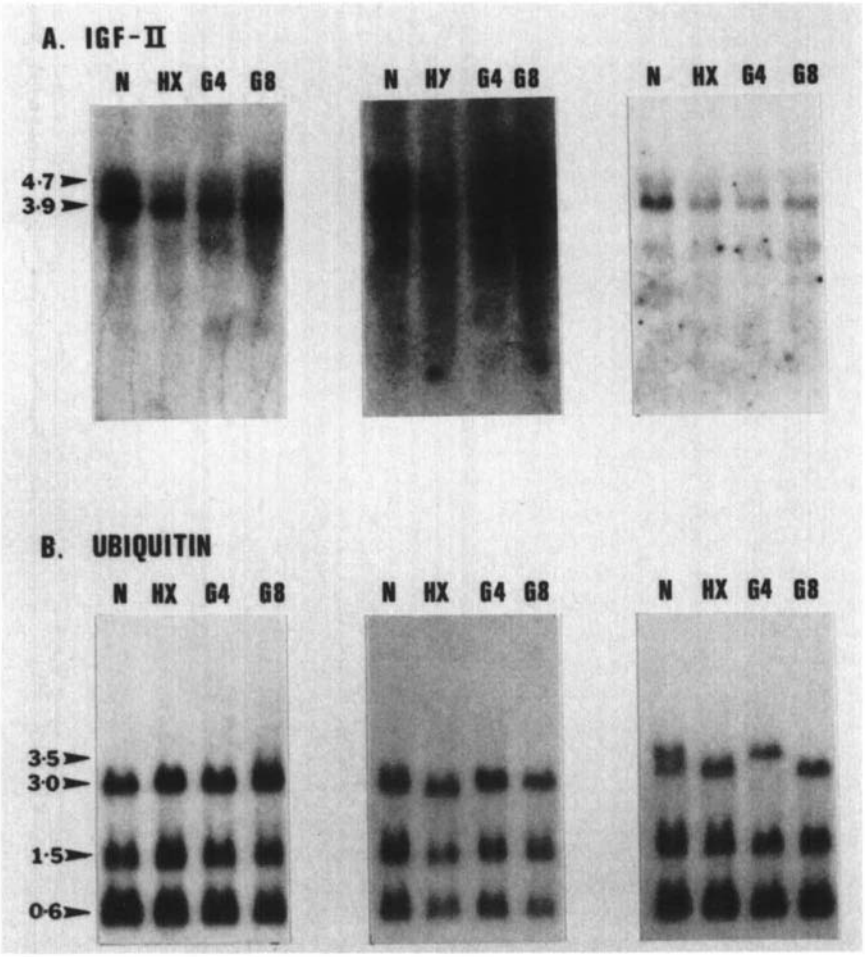


Fig. 5. GH Dependence of Brain IGF-II mRNAs
Autoradiograms of Northern blots of 25 μg poly A^+ RNAs extracted from whole brains of normal (N), hypophysectomized (HX), and hypophysectomized rats 4 h (G4) or 8 h (G8) after ip injection of hGH. *Numbers at the left* of the blots are the estimated sizes of hybridizing mRNAs based on comparison with denatured *Hin*dIII fragments of λ DNA electrophoresed on the same gels as the RNAs. A, Blots were hybridized with a human IGF-II cDNA probe and exposed to x-ray film for 48 h. B, Blots shown in A were stripped of hybridized IGF-II cDNA, rehybridized with a human ubiquitin cDNA probe, and exposed to x-ray film for 24 h.

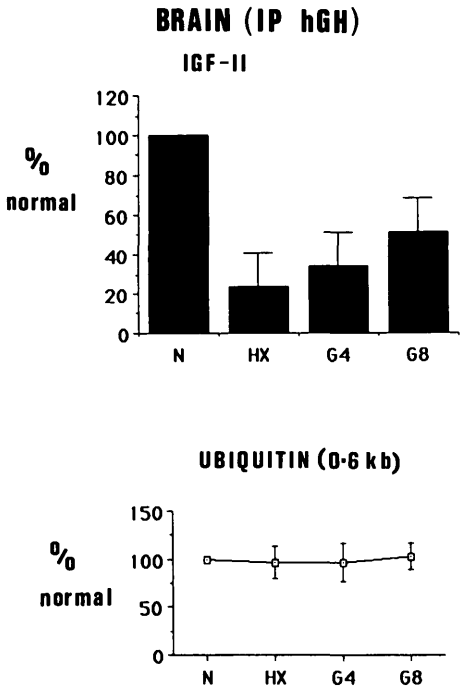


Fig. 6. Abundance of Brain IGF-II mRNAs
The histogram at the top shows the abundance of IGF-II mRNAs in poly A^+ RNAs from whole brain of normal (N), hypophysectomized (HX), and hypophysectomized rats 4 h (G4) or 8 h (G8) after ip injection of hGH. Mean abundance of the 0.6 kb ubiquitin mRNA is plotted at the bottom for comparison. Abundance in each case is expressed as percentage of signal intensity observed in poly A^+ RNAs from normal brain. Each estimate of mRNA abundance is the mean ± SD of three different poly A^+ RNA preparations from three different animals. Statistical comparisons of mRNA abundance are described in the text.

rationale that a rat probe would be more sensitive than a human probe for detection of low abundance Sm-C/IGF-I mRNAs. By use of the rat probe, a 7.5 kb Sm-C/IGF-I mRNA was detected in brain poly A^+ RNAs (Fig. 7) and densitometric scanning (Fig. 8) revealed that the abundance of this mRNA in untreated hypophysectomized rats was 4-fold lower than normal ($P < 0.01$). In hypophysectomized rats given an icv injection of GH 4 h before killing, the abundance of the 7.5 kb brain Sm-C/IGF-I mRNA was higher than in untreated hypophysectomized rats ($P < 0.01$) and was not significantly different from normal. The abundance of the 7.5 kb brain Sm-C/IGF-I mRNA was lower than normal in rats given icv hGH at 8 h before killing ($P < 0.01$) and was not significantly different from the abundance in untreated hypophysectomized rats.

In this second series of experiments brain IGF-II mRNA abundance in untreated hypophysectomized rats was 4-fold lower than normal ($P < 0.01$). The abundance in hypophysectomized rats given icv hGH 4 h before killing also was lower than normal ($P < 0.01$) and did not differ significantly from abundance in the untreated animals. Hypophysectomized animals given icv hGH at 8 h before killing, however, showed higher abundance of brain IGF-II mRNAs than untreated hypophysectomized animals ($P < 0.01$) although the abundance was still only 50% of normal ($P < 0.01$ compared with normal). The abundance of the 0.6 kb brain ubiquitin mRNA did not vary significantly with GH status and served to validate the observed effects of GH status on abundance of brain Sm-C/IGF-I and IGF-II mRNAs.

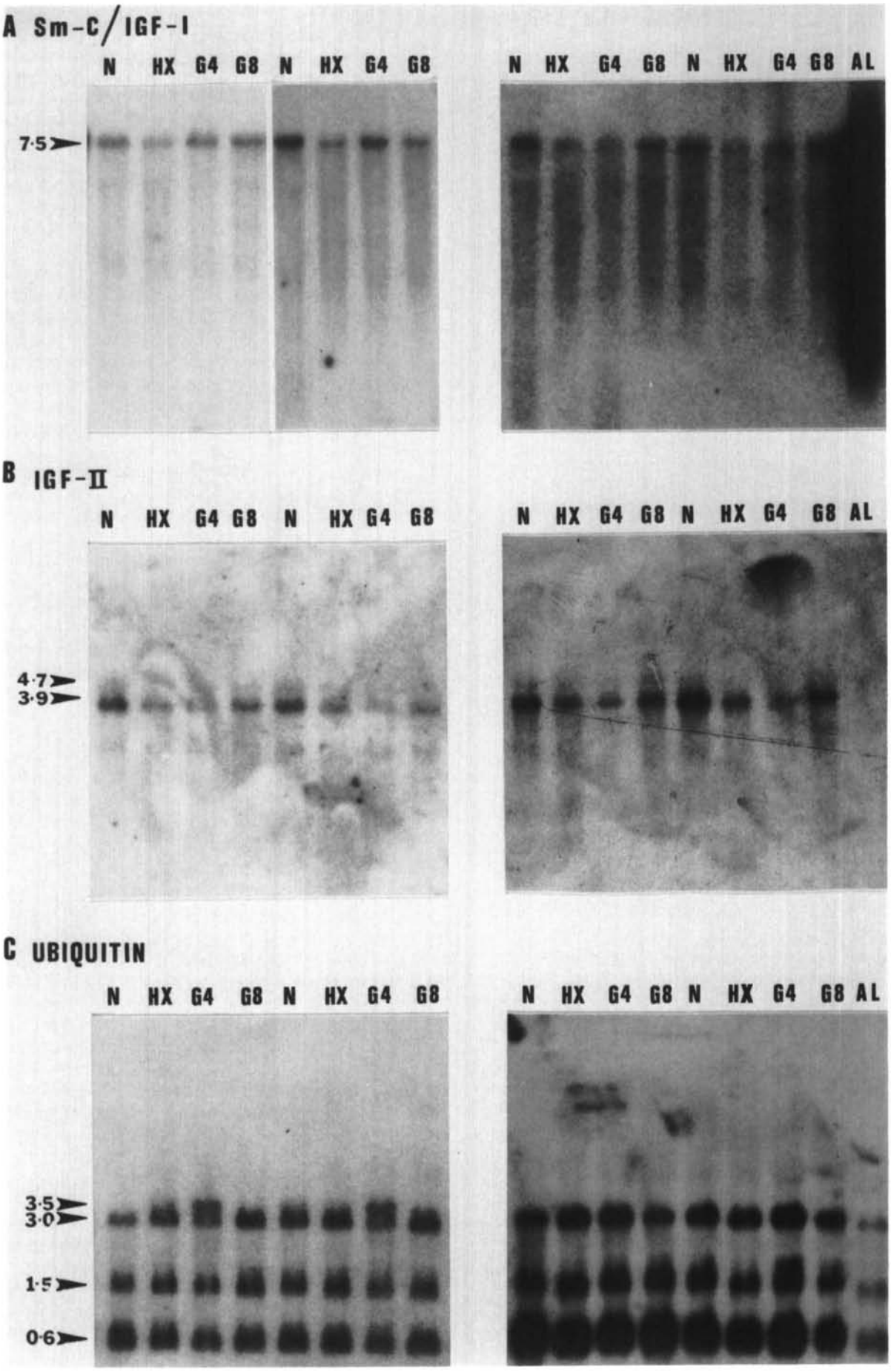
DISCUSSION

The GH dependence of immunoreactive Sm-C/IGF-I concentrations in rat serum and liver is well established (1, 2). Previous studies have shown that immunoreactive Sm-C/IGF-I concentrations are 8 times lower than normal in liver from hypophysectomized rats and are restored to normal within 12 h after GH (4). The present findings demonstrate similar effects of hypophysectomy and GH replacement on the abundance of liver Sm-C/IGF-I mRNAs. Abundance of Sm-C/IGF-I mRNAs was 8- to 10-fold lower in liver from hypophysectomized animals compared with normals and was restored to normal levels in liver from hypophysectomized animals injected with hGH 4 or 8 h before killing.

GH regulation of liver Sm-C/IGF-I mRNAs was more rapid than observed previously for induction of the peptide, thus suggesting that the effects of GH on liver Sm-C/IGF-I concentrations (4) are mediated by a regulation of the rate of transcription of the Sm-C/IGF-I gene and/or Sm-C/IGF-I mRNA stability. Four different liver Sm-C/IGF-I mRNAs of estimated sizes 7.5, 4.7, 1.7, and 1.2 kb were each regulated by GH status suggesting that regulation of all four mRNAs contributes to the effects of hypophysectomy and GH replacement on rat liver Sm-C/IGF-I levels and secretion (4, 5). The structural relationships among the different rat Sm-C/IGF-I mRNAs are not known, but several Sm-C/IGF mRNAs also have been reported in human liver (8). Two of these human Sm-C/IGF-I mRNAs are splicing variants encoding identical Sm-C/IGF-I sequences but with differences in coding sequences for carboxyl-terminal trailer peptides and in 3′-untranslated regions (8). The multiple Sm-C/IGF-I mRNAs observed in rat liver may also be splicing variants of the same Sm-C/IGF-I gene transcript.

IGF-II mRNAs were barely detectable in poly A^+

Fig. 7. GH Dependence of Brain Sm-C/IGF-I and IGF-II mRNAs
Autoradiograms of Northern blots of 25-μg samples of poly A^+ RNAs extracted from whole brain of normal (N), hypophysectomized (HX), and hypophysectomized rats 4 h (G4) or 8 h (G8) after injection of 200 ng hGH into the lateral ventricle (icv injection). AL is 10 μg poly A^+ RNA from rat liver, applied to the blot for comparison. *Numbers at the left* of the blots are estimated sizes of hybridizing mRNAs based on comparison with denatured *Hin*dIII fragments of λ DNA electrophoresed on the same gels as the RNAs. A, Blots were hybridized with a rat Sm-C/IGF-I genomic DNA probe and exposed to x-ray film for 48 h. B, Blots were hybridized with human IGF-II cDNA probe and exposed to x-ray film for 48 h. C, The blots shown in B were stripped for hybridized IGF-II cDNA, rehybridized with the human ubiquitin cDNA, and exposed to x-ray film for 24 h (*left*) and 48 h (*right*).



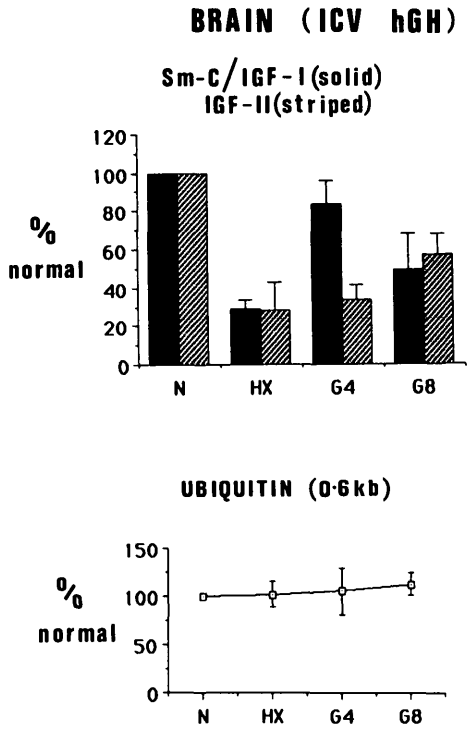


Fig. 8. Abundance of Brain Sm-C/IGF-I and IGF-II mRNAs after Hypophysectomy and icv GH

The histograms at the *top* show the abundance of the 7.5 kb Sm-C/IGF-I mRNA (■) and IGF-II mRNAs (▨) in poly A^+ RNAs from brains of normal (N) hypophysectomized (HX) and hypophysectomized rats 4 h (G4) or 8 h (G8) after icv hGH. Abundance of a 0.6 kb ubiquitin mRNA is shown at the *bottom* for comparison. Abundance in each case is expressed as percentage of signal intensity observed in poly A^+ RNAs from normal brain. Each estimate of mRNA abundance shown is the mean and SD of four different poly A^+ RNA preparations from four different animals. Statistical comparisons of mRNA abundance are described in the text.

RNAs from the rat livers used in this study. Only minor amounts of 4.7 and 3.9 kb IGF-II mRNAs were detected and this is consistent with reports by others of relatively low serum IGF-II levels postnatally (9, 10). We found no evidence for regulation of liver IGF-II mRNAs by GH status which is consistent with previous findings that IGF-II concentrations in serum (1, 6) and conditioned media from rat fibroblasts (7) are less GH dependent than Sm-C/IGF-I.

Our observations of four hybridizing Sm-C/IGF-I mRNAs in poly A^+ RNAs from adult rat pancreas are in agreement with our recent observations that multiple tissues in the adult rat contain Sm-C/IGF-I mRNAs and provide direct support for previous suggestions, that Sm-C/IGF-I is synthesized in tissues other than liver (4). The lower abundance of Sm-C/IGF-I mRNAs found in this study in pancreas compared with liver, and our previous findings of 10- to 50-fold higher levels of Sm-C/IGF-I mRNAs in liver than other tissues in adult rats (11), provide support for the contention that the liver is a major site of Sm-C/IGF-I production (1, 2, 5).

Pancreatic poly A^+ RNAs from hypophysectomized rats contained 5-fold lower levels of Sm-C/IGF-I mRNAs than normal rats. Hypophysectomized rats injected ip with hGH, showed higher levels of Sm-C/IGF-I mRNAs than untreated rats, with restoration to normal levels within 4 h after hGH injection. These findings of GH-dependent regulation of Sm-C/IGF-I mRNAs in pancreas provide evidence that Sm-C/IGF-I biosynthesis is regulated by GH status in other tissues than the liver, and are consistent with previous observations that immunoreactive Sm-C/IGF-I concentrations are regulated by GH in many rat tissues (4). In contrast to our findings with Sm-C/IGF-I mRNAs in adult pancreas, Romanus *et al.* (12) found no clear evidence that biosynthesis of immunoreactive Sm-C/IGF-I is regulated by GH in cultured neonatal rat islets. These different findings may be attributable to the different systems used in the two studies as the regulation of Sm-C/IGF-I biosynthesis by GH may differ in neonatal and adult pancreatic islets, in endocrine and exocrine pancreas or in cultured pancreatic tissue and the pancreas *in vivo*. Further investigations are necessary to resolve these issues.

In pancreas, IGF-II mRNAs were below the detection limit of the hybridization procedures which suggests that IGF-II is not synthesized to a significant extent in adult rat pancreas. This finding is consistent with a previous report that IGF-II is not detectable in cultured neonatal rat islets (12).

In brain, Sm-C/IGF-I mRNAs were not detected when a human Sm-C/IGF-I cDNA was used as probe which is consistent with our previous findings (11). Use of a recently characterized rat Sm-C/IGF-I genomic DNA probe revealed a 7.5 kb Sm-C/IGF-I mRNA in rat brain that was reduced in abundance by hypophysectomy and was restored to normal within 4 h after administration of GH into the lateral ventricle. These findings suggest that GH can regulate Sm-C/IGF-I mRNAs in brain as well as in peripheral tissues. Within 8 h after icv hGH administration, the abundance of brain Sm-C/IGF-I mRNA was similar to that in untreated hypophysectomized animals which could reflect a short half-life of GH in cerebrospinal fluid.

Two IGF-II mRNAs of 4.7 and 3.9 kb were detected in rat brain and showed a 4- to 5-fold reduction in abundance in hypophysectomized animals compared with normal animals. We found evidence for an increase in abundance of brain IGF-II mRNAs by GH treatment of hypophysectomized animals, but the increase occurred only at 8 h after GH treatment, which was slower than the observed GH-dependent increases in brain Sm-C/IGF-I mRNA as well as Sm-C/IGF-I mRNAs in liver and pancreas. The increase in brain IGF-II mRNAs at 8 h after GH treatment was statistically significant, however, only when hGH was administered directly into the lateral ventricle, and not when administered by ip injection. This difference probably reflects a larger variation in replicate estimates of brain IGF-II mRNA abundance in the animals given ip hGH injections rather than an effect of the route of hGH administration.

Our findings suggest that there are independent mechanisms that mediate GH regulation of brain Sm-C/IGF-I and IGF-II mRNAs because Sm-C/IGF-I mRNA

abundance was restored to normal in hypophysectomized animals with 4 h after injection of hGH into the lateral ventricle, while brain IGF-II mRNAs were increased to only 50% of normal at 8 h after icv hGH injection. The less dramatic GH-dependent rise in brain IGF-II mRNAs, compared with Sm-C/IGF-I mRNAs, could, for example, suggest regulation of IGF-II mRNAs only in specific brain regions. Whatever the regulatory mechanisms, our findings of GH-dependent regulation of mRNAs for Sm-C/IGF-I and IGF-II in brain, together with evidence that the Sm/IGFs may regulate neuronal differentiation and axonal and dendritic outgrowth (13), suggest that further studies of the sites of synthesis of the brain Sm/IGFs and the mechanisms by which their synthesis and release is regulated, will aid in our understanding of the biological role of these growth factors in the brain.

MATERIALS AND METHODS

Animals and Tissues

Age matched, normal, and hypophysectomized male Sprague Dawley rats were purchased from Zivic-Miller Labs (Allison Park, PA). Rats were fed standard laboratory chow and provided water, each liter of which contained 2.03 g NaCl, 83.3 mg KCl, 2.1 mg $MgCl_2$, and 50 g sucrose, *ad libitum*.

In a first series of experiments, rats hypophysectomized for 4 weeks were given an ip injection of 200 μg hGH (Crescormon, Kabi, Stockholm, Sweden) in 200 μl 0.9% saline and were then killed by decapitation at 4 h or 8 h after hGH injection. Normal and untreated hypophysectomized rats were killed at the same time as the hypophysectomized rats treated with hGH for 4 h. Completeness of hypophysectomy was verified by direct examination of the sella turcica and by reduced weight gain of hypophysectomized rats compared with normal rats. Weights at the time of killing were 256.3 ± 5.8 g (mean ± SD) for five normal rats, and 113.6 ± 6.4 g for 15 hypophysectomized rats. Within minutes of killing, liver, pancreas, and brain were removed from the animals, frozen in liquid nitrogen, and stored at −100 C until RNA was extracted.

In a second series of experiments, normal rats and rats hypophysectomized 2 weeks previously were anesthetized with nembutal (60 mg/kg). A 23-gauge stainless steel cannula was stereotaxically implanted in the right lateral ventricle of each animal and fixed to the skull with dental cement. Two groups of four hypophysectomized rats were given injections of 200 ng hGH in 10 μl saline through the implanted cannula (icv injection) and were killed 4 h or 8 h later. Normal and hypophysectomized control animals received 10 μl saline icv and were killed 4 h later. Verification of cannula placement was assessed by icv injection of 5 μl trypan blue immediately before killing. Only animals with dye present in the fourth ventricle were used for brain RNA extraction. As with the first series of animals, complete hypophysectomy was verified by examination of sella turcica and reduced weight gain of hypophysectomized animals compared with controls. Weights were 212 ± 8.9 g, for four normal rats and 79 ± 6.2 for 12 hypophysectomized rats.

Preparation of Poly A^+ RNA

Frozen rat tissues were homogenized in 4 M guanidine thiocyanate as described in (14). Total RNA was pelleted by centrifugation of the homogenate over a cushion of 5.7 M CsCl (15). Poly A^+ RNA was purified from total RNA by a modification of the oligo dT cellulose affinity chromatography procedure of Aviv and Leder (16) that is described in detail (11)

Hybridization Probes

In the first series of experiments, Sm-C/IGF-I mRNAs were analyzed with a human Sm-C/IGF-I CDNA (17). A 2.3 kb rat Sm-C/IGF-I genomic DNA probe containing coding sequences for the trailer peptide of the rat Sm-C/IGF-I precursor and 3′-untranslated region was characterized during the course of these studies (unpublished data) and was used as a probe for brain Sm-C/IGF-I mRNAs in the second series of experiments. A human IGF-II cDNA probe (18) was used in all experiments. A human ubiquitin cDNA probe (19) was used as a control probe. DNA probes were labeled to specific activities of 10^7–10^8 cpm/μg by nick translation (20).

Northern Blot Hybridization Analyses

Poly A^+ RNAs were denatured in glyoxal and dimethylsulfoxide and size fractionated on 1% agarose gels (21). After electrophoresis, samples were transferred to gene screen (New England Nuclear, Boston, MA) by the blotting method of Southern (22). Hybridization was in a buffer containing 50% deionized formamide, 50 mM Tris-HCl, pH 7.0, 6X SSC, 5X Denhardts, 0.1% sodium dodecyl sulfate (SDS) and 100 μg/ml sonicated denatured salmon sperm DNA (20X SSC = 3 M NaCl, 0.3 M Na Citrate, pH 7.0; 20X Denhardts = 0.4% each BSA, ficoll, and polyvinylpyrrolidone). Hybridization was for 48 h at 42 C with 0.5×10^6 cpm/ml of labeled probe. Hybridized blots were washed successively for 60 min each in 2X SSC, 0.5% SDS at room temperature, 2X SSC, 1% SDS at 65 C, and 0.1X SSC at 60 C. Blots were then air dried and exposed to x-ray film (Kodak XAR-5) at −100 C with intensifying screens. Control hybridizations with ubiquitin cDNA probe were performed on replicate blots of poly A^+ RNAs or on blots that had been hybridized with Sm/IGF probes then stripped of hybridized probe by washing in 0.1X SSC at 100 C. Dot blots of the poly A^+ RNAs extracted from liver were prepared, and were hybridized and washed in the same way as Northern blots.

Data Analyses and Presentation

Poly A^+ RNA preparations were prepared and analyzed in groups of four, each containing a tissue sample from a normal rat, an untreated hypophysectomized rat, and from two hypophysectomized rats, one treated with hGH 4 h before killing and the other treated at 8 h before killing. The abundance of hybridizing Sm-C/IGF-I, IGF-II, and ubiquitin mRNAs in each group of four preparations was estimated by densitometric scanning (Darwin densitometry system, Darwin Instruments, Inc., Winston Salem, NC) of autoradiograms of Northern blots or dot blots. As human Sm-C/IGF-I and IGF-II cDNAs were used as probes to quantify the corresponding rat mRNAs in most of the hybridizations, no attempt was made to assess absolute abundance of the rat Sm/IGF mRNAs. Rather, for each group of four poly A^+ preparations analyzed, the hybridization signal intensity for Sm/IGF mRNAs in the normal tissue sample was assigned a value of 100% and signal intensities in samples from untreated and hGH-treated hypophysectomized rats were expressed as a percentage of normal. For each tissue studied and each experimental condition, the abundance of Sm/IGF mRNAs was assessed in poly A^+ RNAs prepared from at least three different animals. The replicate estimates of Sm/IGF mRNA abundance in tissues from normal, hypophysectomized, and hypophysectomized and hGH-treated rats were compared by analysis of variance with multiple comparisons by Tukey's test (23). Statistical significance was set at $P < 0.05$.

Acknowledgments

We would like to thank Eileen Hoyt for invaluable technical assistance.

Received June 30, 1986.

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* This work was supported in part by NIH Grants R01-AM-1022 (to J.J.v.W.), R01-HD-08299 (to A.J.D.), GM-35734 (to P.K.L.), Career Research Award AM-14115 (to J.J.v.W.), and Career Research Development Award HD-00435 (to A.J.D.).

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