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ORIGINAL PAPER

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The effect of hypophysectomy on pancreatic islet hormone and insulin-like growth factor I content and mRNA expression in rat

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Abstract The growth arrest after hypophysectomy in rats is mainly due to growth hormone (GH) deficiency because replacement of GH or insulin-like growth factor (IGF) I, the mediator of GH action, leads to resumption of growth despite the lack of other pituitary hormones. Hypophysectomized (hypox) rats have, therefore, often been used to study metabolic consequences of GH deficiency and its effects on tissues concerned with growth. The present study was undertaken to assess the effects of hypophysectomy on the serum and pancreatic levels of the three major islet hormones insulin, glucagon, and somatostatin, as well as on IGF-I. Immunohistochemistry (IHC), in situ hybridization (ISH), radioimmunoassays (RIA), and Northern blot analysis were used to localize and quantify the hormones in the pancreas at the peptide and mRNA levels. IHC showed slightly decreased insulin levels in the β cells of hypox compared with normal, age-matched rats whereas glucagon in α cells and somatostatin in δ cells showed increase. IGF-I, which localized to α cells, showed decrease. ISH detected a slightly higher expression of insulin mRNA and markedly stronger signals for glucagon and somatostatin mRNA in the islets of hypox rats. Serum glucose concentrations did not differ between the two groups although serum insulin and C-peptide were lower and serum glucagon was higher in the hypox animals. These changes were accompanied by a more than tenfold drop in serum IGF-I. The pancreatic insulin content per gram of tissue was not significantly different in hypox and normal rats. Pancreatic glucagon and somatostatin per gram of tissue were higher in the hypox animals. The pancreatic IGF-I content of hypox rats was significantly reduced. Northern blot analysis gave a 2.6-, 4.5-, and 2.2-fold increase in pancreatic insulin, glucagon, and somatostatin mRNA levels, respectively, in hypox rats, and a 2.3-fold decrease in IGF-I mRNA levels. Our results show that the fall of serum IGF-I after hypophysectomy is accompanied by a decrease in pancreatic IGF-I peptide and mRNA but by partly discordant changes in the serum concentrations of insulin and glucagon and the islet peptide and/or mRNA content of the three major islet hormones. It appears that GH deficiency resulting in a "low IGF-I state" affects translational efficiency of these hormones as well as their secretory responses. The maintenance of normoglycemia in the presence of reduced insulin and elevated glucagon serum levels, both of which would be expected to raise blood glucose, may result mainly from the enhanced insulin sensitivity, possibly due to GH deficiency and the subsequent decrease in IGF-I production.

Keywords Insulin · Glucagon · Somatostatin · Growth hormone · IGF-I · Metabolism · Hypophysectomy

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Introduction

Growth hormone (GH) is the major regulator of postnatal growth. The removal of the pituitary gland in young growing rats leads to growth arrest. This appears to be mainly caused by the lack of GH because replacement of GH (Simpson et al. 1949; Guler et al. 1989) or insulin-like growth factor (IGF) -I (Guler et al. 1989) leads to resumption of growth despite the lack of other pituitary hormones. Hypophysectomized (hypox) rats therefore lend themselves to study the consequences of GH deficiency on tissues concerned with growth and

on metabolism. However, studies dealing with the effects of hypophysectomy on pancreatic hormones are sometimes difficult to compare and/or are partly controversial. Thus, the insulin content per gram of tissue in the pancreas of hypox rats was found to be unchanged but decreased when expressed per whole pancreas (Malaisse et al. 1968). The insulin content expressed per islet of cultured islets from hypox rats was reported to be unchanged (Pierluissi et al. 1980). In contrast, islets isolated and cultured 4 weeks after hypophysectomy contained less insulin per microgram of protein than islets from normal rats (Martin and Gagliardino 1967). Pancreatic somatostatin per gram of tissue was found to be unchanged (Berelowitz et al. 1981) whereas Recant et al. (1984) found elevated somatostatin whether expressed per milligram of protein or per whole pancreas of hypox rats. To our knowledge, no data are available regarding pancreatic glucagon content in hypox rats. Serum glucagon content in hypox rats has been reported in only one study, where it was found to be elevated (Van Lan et al. 1974). Furthermore, we are not aware of any data on islet hormone gene expression in the pancreas of hypox rats. Above all, no study has investigated the consequences of hypophysectomy in rats on the three major pancreatic hormones insulin, glucagon, and somatostatin, simultaneously at the peptide and mRNA levels and compared immunohistochemical (IHC) with radioimmunological determinations or in situ hybridization (ISH) with Northern blot analysis. Therefore, the aim of our study was to combine these methodologies in order to look for potential changes of these pancreatic hormones following hypophysectomy in rats and to localize these changes within the pancreatic islets.

Materials and methods

Animals

The experiments were approved by the Institutional Animal Welfare Committee. Seven-week-old, normal ($n = 6$), hypophysectomized (hypox; $n = 6$) male Wistar rats (150–160 g) were obtained from Charles River Laboratories (Charles River, Iffa Credo, France). The rats were kept at 25°C on a 12-h light/12-h dark cycle and had free access to food and drinking water.

Tissue sampling

Two weeks after hypophysectomy, the rats were anesthetized with pentothal (Abbott Laboratories S.A, Baar, Switzerland) and bled by aortic puncture. Blood samples were collected on ice and, after clotting, centrifuged for 30 min at 3,000 *g* at 4°C. The serum was stored at −20°C for further analysis. The pancreas was excised, weighed, and divided into three parts for (1) fixation for ISH and immunohistochemistry, (2) RNA extraction, and (3) peptide extraction. Pancreas specimens were fixed in Bouin's solution without acetic acid for 4 h and embedded in paraffin. Total RNA was extracted immediately after removal of the tissue using the extraction solution provided with the RNA extraction kit (NucleoSpin RNA II, Machery-Nagel, Düren, Germany) and stored at −80°C until use. Tissue for protein extraction was stored at −80°C until use.

Immunohistochemical protocol

Sections were cut at 4 μm, deparaffinized in xylol, rehydrated through a series of descending concentrations of ethanol, and rinsed in deionized water. Nonspecific binding was reduced by treatment of the sections with phosphate buffered saline (PBS) containing 2% bovine serum albumin (BSA) for 30 min at room temperature. Thereafter, the sections were incubated with rabbit antisera directed against porcine glucagon (code A565, 1:2000; Dako, Carpinteria, CA, USA), human (h) somatostatin (code AO566, 1:300; Dako), or hIGF-I (Maake and Reinecke 1993; Zapf et al. 2002; 1:300), respectively, for 18 h at 4°C. After rinsing in PBS/2% BSA, the antisera were visualized by incubation for 2 h at room temperature with fluorescein-isothiocyanate (FITC)-labeled goat antirabbit IgG (1:100; Dako). For the detection of insulin, sections were incubated for 18 h at 4°C with a guinea pig antiserum against porcine insulin (code A564; 1:18,000; Dako), washed three times with PBS/2% BSA, and treated with biotinylated goat antiguinea pig IgG (1:100; Bioscience, Emmenbrücke, Switzerland) followed by FITC-coupled streptavidin (1:1000; Bioscience) for 30 min at room temperature. For control incubations, the primary antisera were replaced by nonimmune sera or preabsorbed with the respective antigens (0.4–40 μg peptide/ml diluted antiserum). The sections were mounted with glycergel (Dako), examined, and photographed with a Zeiss axioscope (Zeiss, Zürich, Switzerland).

Preparation of digoxigenin (DIG)-labeled RNA probes

Pancreatic total RNA from normal rats was, in a first step, reverse transcribed by M-MLV reverse transcriptase (Promega, Madison, WI, USA) in the presence of oligo (dT) primer and 1× reaction buffer (5× = 250 mM Tris–HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$, and 50 mM DTT) to generate cDNA. For the generation of the insulin cDNA probe, cDNA was subjected to polymerase chain reaction (PCR) using the sense primer 5′-CAGCACCTTTGTGGTTCTCA-3′ and the antisense primer 5′-AGGGTAGTGGTGGGCCTAGT-3′, corresponding to exon 2 and 3 in the rat insulin 2 gene (GenBank Accession no. V01243 C06616). PCR was performed in a GeneAmp PCR System 9600 cycler (Perkin Elmer, Norwalk, CT, USA) in 1.5 mM $MgCl_2$, 0.2 μM of each primer, 200 μM of each dNTP, and 1U of Taq polymerase (Qbiogene Inc., Basel, Switzerland), as recommended by the supplier. Amplification condi-

tions were optimized to reach linear PCR signals, i.e., one cycle of 5 min at 95°C, 45 s at 58°C, and 1 min at 72°C; 33 cycles of 45 s at 95°C, 45 s at 58°C, and 1 min at 72°C followed by a final extension step of 5 min at 72°C. The PCR product was then analyzed by electrophoresis on 1.5% agarose gel and purified using a PCR Purification Kit (Qiagen, Basel, Switzerland), according to the manufacturer's instructions. The rat insulin cDNA probe (267-bp fragment), thus obtained, was then cloned in a PCR-Script SK (+) cloning vector that contained a T7 RNA polymerase binding site adjacent to the multiple cloning site using a commercial kit (Stratagene, Heidelberg, Germany). The plasmid containing the insulin cDNA fragment was linearized by restriction enzyme Not I (Promega). Thereafter, the insulin RNA probe was synthesized by in vitro transcription using DIG RNA labeling kit and T7 RNA polymerase (Roche Inc., Rotkreuz, Switzerland). For the generation of glucagon and somatostatin cDNA probes, the cDNA was subjected to PCR using specific primers for glucagon (sense primer 5′-AACGAAGACAAACGCCATTC-3′ and antisense primer 5′-GCCCAAGTTCCTCAGCTATG-3′, corresponding to the nucleotide sequence 138–428, GenBank Accession no. NM 012707), and somatostatin (sense primer 5′-TGGCAGAACTGCTGTCTGAG-3′ and antisense primer 5′-AACGCAGGGTCTAGTTGAGC-3′, corresponding to the nucleotide sequence 218–517, GenBank Accession no. NM 012659) whereby the 5′ end of the antisense primers contained an additional sequence encoding for T7 RNA polymerase promoter (Microsynth, Balgach, Switzerland). PCR amplification conditions were optimized to reach linear PCR signals, as follows: one cycle of 1 min at 95°C, 45 s at 59°C (glucagon) or 61°C (somatostatin), and 1 min at 72°C; 33 cycles of 45 s at 95°C, 45 s at 59°C (glucagon) or 61°C (somatostatin), and 1 min at 72°C followed by a final extension step of 5 min at 72°C. The PCR products were analyzed by electrophoresis on 1.5% agarose gel and purified using a PCR Purification Kit (Qiagen). For synthesis of the DIG-labeled RNA probes, 200 ng of the glucagon and somatostatin PCR products, respectively, were in vitro transcribed using the DIG RNA labeling kit and T7 RNA polymerase (Roche). Integrity of the probes and efficiency of DIG-labeling were confirmed by gel electrophoresis and dot blot.

In situ hybridization protocol

Paraffin sections cut at 4 μm were mounted on SuperFrost Plus slides (Menzel-Gläser, Germany), and dried overnight at 42°C. After dewaxing in xylol and rehydration, the sections were postfixed with 4% paraformaldehyde and 0.1% glutaraldehyde in 1× PBS. The following steps were carried out with diethylpyrocarbonate (DEPC)-treated solutions (DEPC, Sigma, Switzerland) in a humidified chamber: Sections were digested with 0.02% proteinase K in 20 mM Tris–HCl, pH 7.4 containing 2 mM $CaCl_2$ for 10 min at 37°C to denature proteins. Background reduction was achieved by treatment of the sections with 1.5% triethanolamine and 0.25% acid anhydride for 10 min at room temperature. The slides were incubated with 100 μl prehybridization solution (50% formamide, 1× PBS, 2.5× Denhardt's solution, 25 mM EDTA, 275 μg/ml single-strand DNA, and 250 μg/ml yeast transfer RNA) per section for 3 h at 60°C (insulin), 58°C (glucagon), and 54°C (somatostatin). The hybridization was carried out overnight at the same temperatures with 50 μl hybridization buffer containing 50% formamide, 1× PBS, 2× Denhardt's solution, transfer RNA (1.5 μg/ml), single-strand DNA (200 μg/ml), 10 mM dithiothreitol, 20% dextran sulfate, and 200 ng antisense DIG-labeled RNA probe (previously denatured for 5 min at 85°C).

The slides were washed for 15 min at room temperature in 2× SSC and for 30 min at specific hybridization temperatures at descending concentrations of SSC (2, 1, 0.5, and 0.2×). DIG detection was performed according to the manufacturer's instructions. In brief, after treatment of the sections with 1% blocking reagent (Roche) in 100 mM Tris–HCl, pH 7.4 containing 150 mM NaCl, the alkaline phosphatase-coupled anti-DIG antibody was diluted 1:4,000 in blocking solution, and sections were incubated for 1 h at room temperature in the dark. After washing twice in 100 mM Tris–HCl, pH 7.4 containing 150 mM NaCl for 15 min each time, sections were treated with 100 mM Tris–HCl, pH 9.5 containing 5 mM levamisole, 0.1% gelatin, nitro blue tetrazolium (188 μg/ml), and 5-bromo-4-chloro-3-indolyl-phosphate (375 μg/ml). Color development was performed for 16 h at room temperature. The color reaction was stopped by rinsing the slides in tap water for at least 15 min. Sections were mounted with glycergel. Microscopic images were obtained with a Zeiss axioscope.

Preparation of radioactively labeled cDNA probes

Pancreatic (glucagon and somatostatin cDNA probes) and hepatic (IGF-I cDNA probe) total RNA from normal rats were reverse transcribed, as described earlier, to generate cDNA. For the generation of glucagon and somatostatin cDNA probes, PCR was performed, as already described, using the same primer sequences with the difference that the 5′ end of the antisense primers did not contain a T7 RNA polymerase promoter sequence. For the generation of radioactively labeled IGF-I DNA probes, the sense primer 5′-ACATCATGTCGTCTTCACATC-3′ and the antisense primer 5′-GGTCTTGTTTCCTGCACTTC-3′, spanning the nucleotide sequence 38–395 (GenBank Accession no. D00698), were used. Optimized PCR conditions were as follows: one cycle of 1 min at 95°C, 45 s at 60°C, 1 min at 72°C; 35 cycles of 45 s at 95°C, 40 s at 60°C, 1 min at 72°C followed by a final extension step of 5 min at 72°C. The PCR products were analyzed by electrophoresis on 1.5% agarose gel and purified using a PCR Purification

Kit (Qiagen). The rat insulin cDNA probe (500-bp fragment) was kindly provided by Dr. J.H. Nielsen (Hagedorn Research Institute, Gentofte, Denmark). The yeast 18S ribosomal cDNA probe was a kind gift from Dr. Kalousek (University Hospital, Zürich). The rat insulin, glucagon, somatostatin, IGF-I, and yeast 18S ribosomal cDNA probes were labeled by random primer extension using a commercial kit (Boehringer Mannheim, Rotkreuz, Switzerland) and [α-^{32}P] deoxy-CTP (3,000 Ci/mmol; Amersham, Cardiff, UK) to the specific activities of 2–4×10^9 cpm/μg DNA following the manufacturer's instructions.

Northern blotting

Twenty micrograms of total RNA isolated from pancreatic tissue was heat-denatured at 65°C for 15 min. Thereafter, RNA samples were electrophoresed on 1% agarose gel containing 2 M formaldehyde, transferred to nylon membranes (Hybond-N, Amersham) by capillary blotting, and fixed by UV cross-linking. Membranes were prehybridized at 42°C in a solution containing 50% (vol/vol) deionized formamide, 5× Denhardt's solution (Ficoll 0.02% wt/vol, and polyvinyl pyrrolidone 0.02% wt/vol), 5× SSPE (20×: 3.6 M NaCl, 0.2 M sodium phosphate, and 0.02 M EDTA, pH 7.7), 0.2% SDS, and 100 μg/ml heat-denatured salmon sperm DNA. Hybridizations were performed in the same solution with ~2×10^7 cpm ^{32}P-labeled cDNA probe for insulin, glucagon, somatostatin, IGF-I, and 18S cDNA/membrane. After 48 h of incubation at 42°C, the membranes were washed twice for 10 min at room temperature and for 15 min at 54°C (insulin, IGF-I), 51°C (glucagon and somatostatin), or 65°C (18 S) in 0.1× SSC/0.1% SDS. Membranes were then exposed at −80°C to an X-Omat AR-5 film (Kodak, Rochester, NY, USA) in cassettes equipped with intensifying screens to visualize [^{32}P]cDNA-mRNA hybrids. Levels of mRNA were quantified by scanning densitometry using a Bio-Rad video densitometer (Richmond, CA, USA). In between consecutive hybridizations, the membranes were washed with 0.1× SSC/0.1% SDS at 98°C for 45 min to remove the previous cDNA probe. Variations in gel loading were corrected against 18S ribosomal RNA values.

Serum glucose analysis

Serum glucose was determined with a Beckman glucose analyzer (Beckman Coulter, Fullerton, CA, USA).

Tissue extraction for radioimmunoassays

Pancreatic tissue, 50 mg, was homogenized using a Polytron homogenizer (Kinematica, Luzern, Switzerland) in 1 ml ice-cold acidic alcohol (0.18 N HCl/70% EtOH), incubated overnight at 4°C, and centrifuged at 16,000 *g* for 5 min. The supernatants were used for determination of insulin, glucagon, somatostatin and IGF-I contents using specific radioimmunoassay (RIA).

Radioimmunoassays

All RIAs were performed according to manufacturers' protocols. Serum levels were determined in the undiluted samples. Serum and pancreatic insulin and glucagon content and serum C-peptide levels were determined with specific rat RIA kits (Linco Research, Inc., St. Charles, MO, USA). For determination of pancreatic insulin and glucagon contents, the extracts were diluted 1:2,000–1:4,000 in the assay buffer provided. The somatostatin concentrations in pancreatic extracts were determined (dilution 1:500) using a human somatostatin RIA kit (EURO-Diagnostica, Malmö, Sweden). It was not possible to measure serum somatostatin because this assay would have required 5 ml of serum. Serum and tissue IGF-I levels were determined by RIA after SepPak C18 chromatography (Waters Corp., Milford, MA, USA), as described previously (Boni-Schnetzler et al. 1999; Zapf et al. 2002). In brief, for determination of serum IGF-I, 0.15 ml PBS containing 0.2% human serum albumin (HSA), pH 7.4, was added to 0.1 ml serum. For determination of pancreatic IGF-I, 0.5 ml of the extract was lyophilized, dissolved in 0.25 ml of PBS/0.2% HSA, and centrifuged. All samples were acid-treated and run over SepPak C18 cartridges, according to the protocol supplied by Immunonuclear (Stillwater, MN, USA). After reconstitution with 1-ml PBS/0.2% HSA for serum and 0.6 ml PBS/0.2% HSA for pancreatic extracts, samples were assayed at three different dilutions (1:5, 1:10, 1:20 for serum, and undiluted 1:2, 1:4 for pancreatic extracts) (Boni-Schnetzler et al. 1999; Zapf et al. 2002). Then, 0.2 ml of samples or standards (rat IGF-I from GroPep, Adelaide, SA, Australia) and 0.1 ml of IGF-I antiserum (final dilution 1:20,000) were preincubated for 24 h at 4°C. Then, 25,000–35,000 cpm of ^{125}I-IGF-I (Anawa, Wangen, Switzerland, specific activity 300–400 μCi/μl) was added to the final incubation volume of 0.4 ml. The reaction mixture was then incubated for another 24 h before precipitation with the secondary antibody (goat antirabbit gamma globulin antiserum) (Zapf et al. 1981). After centrifugation, the pellet was counted in a gamma counter.

Determination of blood volume in pancreatic tissue

The amount of blood in the pancreas was determined according to Hohorst et al. (1950) and has recently been described by Jevdjovic et al. (2004).

Statistical analysis

All results are expressed as mean ± SEM. Statistical analysis was performed by Student's *t* test (significance level $P < 0.05$) with a StatView 4.5 program (Abacus Concepts, Inc., Berkeley, CA, USA).

Results

Body and pancreatic weight

Normal (age-matched) rats weighed 225 ± 6 g whereas hypox rats weighed 153 ± 4 g (mean ± SD). The mean pancreatic weight of normal rats was 429 ± 48 mg and that of hypox rats was 278 ± 22 mg ($P < 0.0001$).

Distribution of insulin, glucagon, somatostatin, and IGF-I peptide in rat pancreatic islets

Insulin immunoreactivity in the β cells was slightly decreased in the islets of hypox (Fig. 1b) compared to the normal (Fig. 1a) rats whereas glucagon immunoreactivity in the α cells (Fig. 2a) and somatostatin immunoreactivity in the δ cells (Fig. 3a) was more pronounced in hypox rats (Figs. 2b, 3b). IGF-I immunoreactivity was localized to α cells (Fig. 4a) and was lower in islets of hypox rats (Fig. 4b).

Localization of insulin, glucagon, and somatostatin mRNA

In situ hybridization showed more intense signals for insulin (Fig. 1d), glucagon (Fig. 2d), and somatostatin (Fig. 3d) mRNAs in the islets of hypox rats compared with controls (Figs. 1c, 2c, 3c).

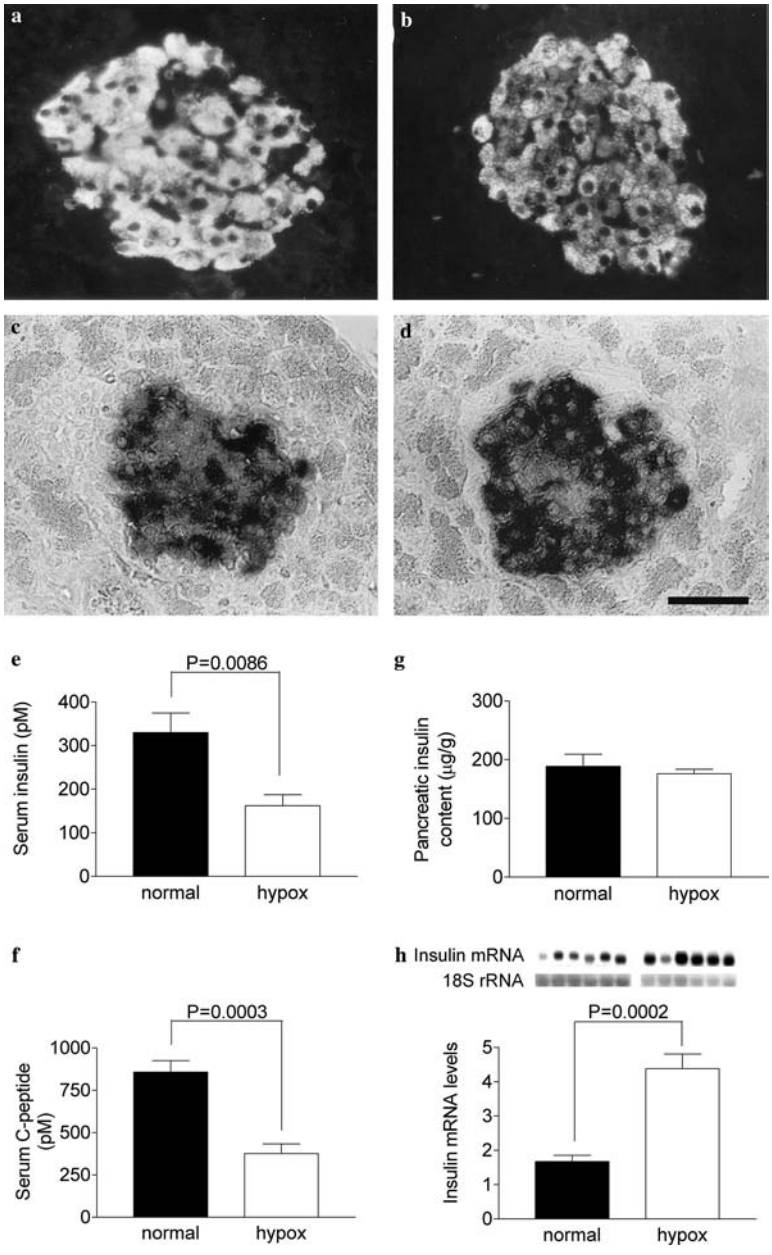

Fig. 1 Localization of insulin peptide (**a**, **b**) and mRNA (**c**, **d**) in normal (**a**, **c**) and hypophysectomized (hypox) (**b**, **d**) rats. Bar: 40 µm. Serum insulin (**e**), C-peptide (**f**), pancreatic insulin peptide content (**g**), and insulin mRNA levels (**h**) of normal and hypox rats. Serum insulin, C-peptide, and pancreatic insulin were measured by specific rat insulin and C-peptide RIA. Insulin mRNA content was determined by Northern blot analysis. Insulin mRNA and 18S rRNA signals are shown above the corresponding columns for each of the two groups (**h**). The insulin mRNA signals were normalized against the18S rRNA signals and expressed as relative levels. *Columns* give the mean values ($n = 6$), and *bars* give the SEM. Significance levels are indicated in **e**, **f**, and **h**

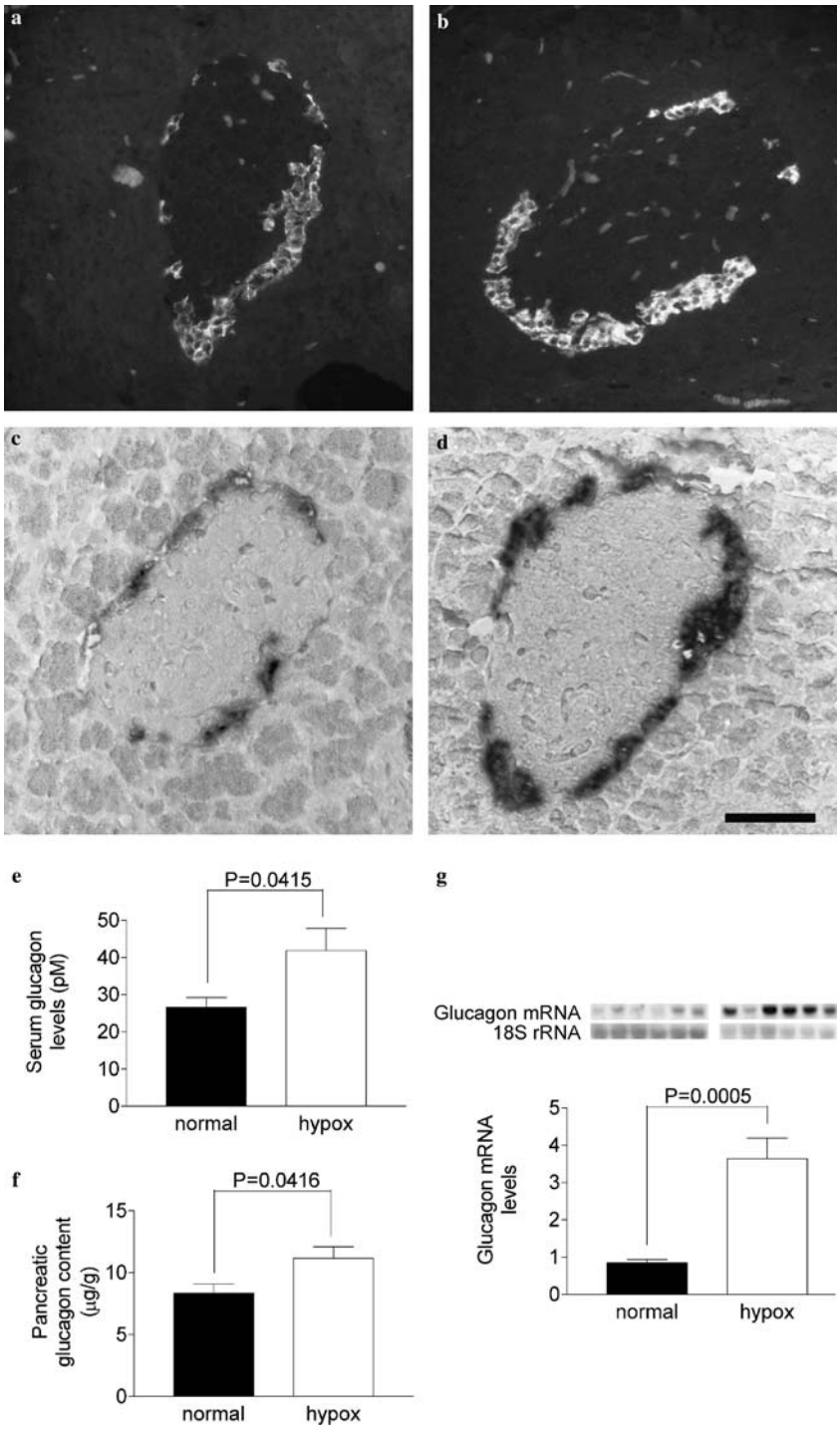

Fig. 2 Localization of glucagon peptide (**a**, **b**) and mRNA (**c**, **d**) in normal (**a**, **c**) and hypophysectomized (hypox) (**b**, **d**) rats. Bar: 60 μm. Serum glucagon (**e**), pancreatic glucagon peptide content (**f**), and glucagon mRNA levels (**g**) of normal and hypox rats. Serum and pancreatic glucagon were measured by a specific rat glucagon RIA, and glucagon mRNA was determined by Northern blot analysis. Northern blot signals for glucagon mRNA and 18S rRNA are shown above the corresponding columns for each of the two groups (**g**). Glucagon mRNA signals were normalized against 18S rRNA signals and expressed as relative levels. *Columns* give the mean values ($n = 6$), and *bars* represent SEM. Significance levels are shown in **e**–**g**

Serum glucose

Serum glucose levels were not significantly different between normal and hypox rats (9.17 ± 0.43 vs. 8.65 ± 0.41 mmol/l, respectively).

Serum insulin and C-peptide concentrations and pancreatic insulin peptide and mRNA levels

Serum insulin concentrations were lower (162 ± 25 pmol/l) in hypox than in normal rats (330 ± 45 pmol/l, $P = 0.0086$; Fig. 1e). The decrease in serum insulin after hypophysectomy was accompanied by a decrease in serum C-peptide concentrations from 858 ± 67 pmol/l to 377 ± 57 pmol/l ($P = 0.0003$; Fig. 1f). There was no significant difference in the amount of insulin per gram of pancreatic tissue between normal and hypox rats, with the values being 188.7 ± 20.7 μg/g and 176.0 ± 7.7 μg/g, respectively (Fig. 1g), but when expressed per whole pancreas, hypox rats had a significantly lower insulin content than normal rats (46.9 ± 2.3

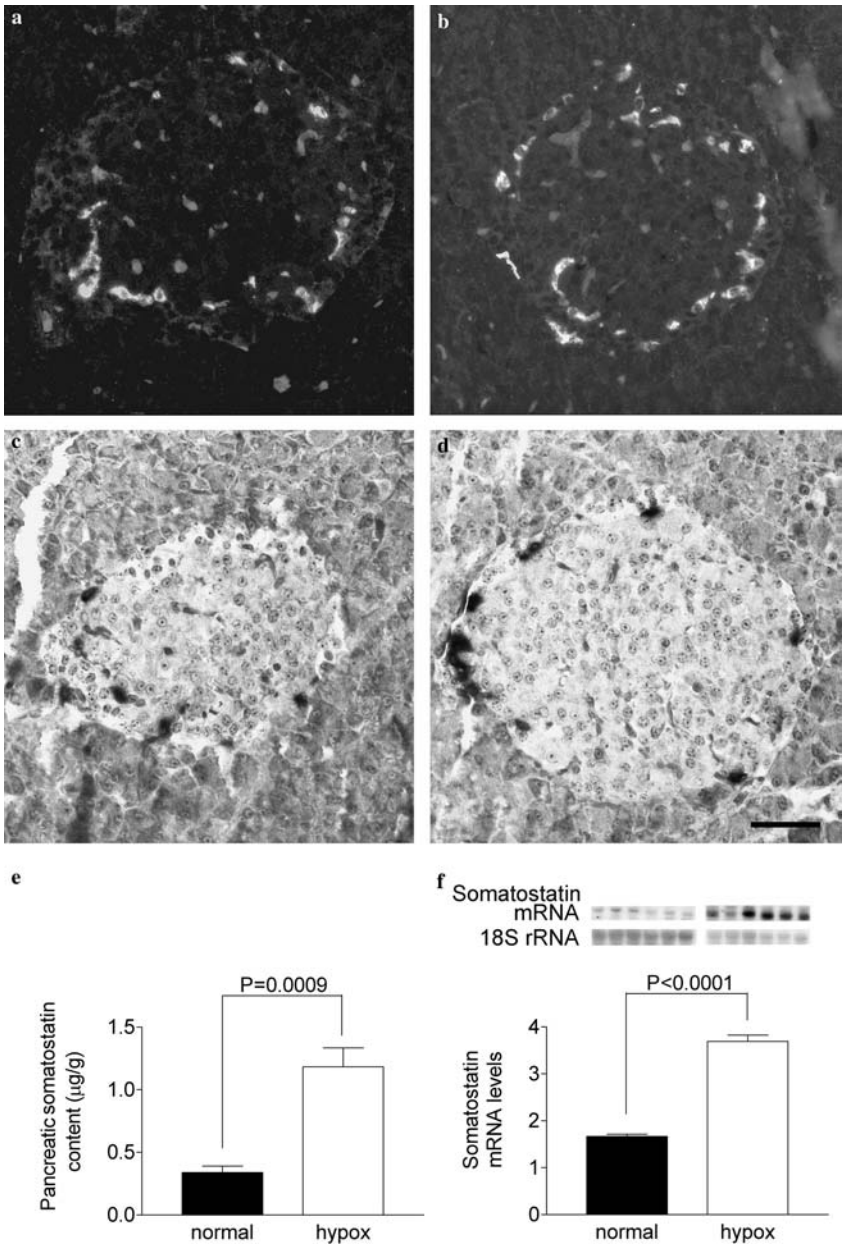

Fig. 3 Localization of somatostatin peptide (**a**, **b**) and mRNA (**c**, **d**) in normal (**a**, **c**) and hypophysectomized (hypox) (**b**, **d**) rats. Bar: 60 µm. Pancreatic somatostatin peptide (**e**) and mRNA (**f**) levels of normal and hypox rats. Pancreatic somatostatin was measured using an RIA against human somatostatin. Somatostatin mRNA was determined by Northern blot analysis. Northern blot signals for somatostatin mRNA and 18S rRNA are shown above the corresponding columns for each of two groups (**f**). Somatostatin mRNA signals were normalized against 18S rRNA signals and expressed as relative levels. *Columns* give the mean values ($n=6$), and *bars* represent SEMs. Significance levels are shown in **e** and **f**

vs. 81.6 ± 10.9 µg per whole pancreas, respectively; $P=0.0197$) due to lower pancreas weights. Northern blot analysis showed a 2.6-fold increase in insulin mRNA expression in the pancreas from hypox compared with normal rats ($P=0.0002$, Fig. 1h).

Serum glucagon concentrations and pancreatic glucagon peptide and mRNA levels

Serum glucagon concentrations were higher in hypox (41.8 ± 6.0 pmol/l) than in normal rats (26.6 ± 2.6 pmol/l; $P=0.0415$; Fig. 2e). Similarly, immunoreactive glucagon per gram of pancreatic tissue was higher in hypox (11.15 ± 0.94 µg/g) than in normal animals (8.34 ± 0.75 µg/g; $P=0.0416$; Fig. 2f). However, there was no statistical difference in the total pancreatic glucagon content per pancreas between hypox and normal animals (3.1 ± 0.3 vs. 3.6 ± 0.3 µg per pancreas, respectively, $P=0.294$). By Northern blot analysis, pancreatic glucagon mRNA levels were found to increase in hypox rats (3.6 ± 0.55) compared with normal rats (0.85 ± 0.08; $P=0.0005$; Fig. 2g).

Pancreatic somatostatin peptide and mRNA levels

The pancreatic somatostatin peptide content was higher in hypox (1.18 ± 0.15 µg/g) than in normal rats (0.34 ± 0.05 µg/g; $P=0.0009$; Fig. 3e), whether expressed per gram of tissue (1.18 ± 0.15 µg/g vs. 0.34 ± 0.05 µg/g) or per whole pancreas (333 ± 50 vs.

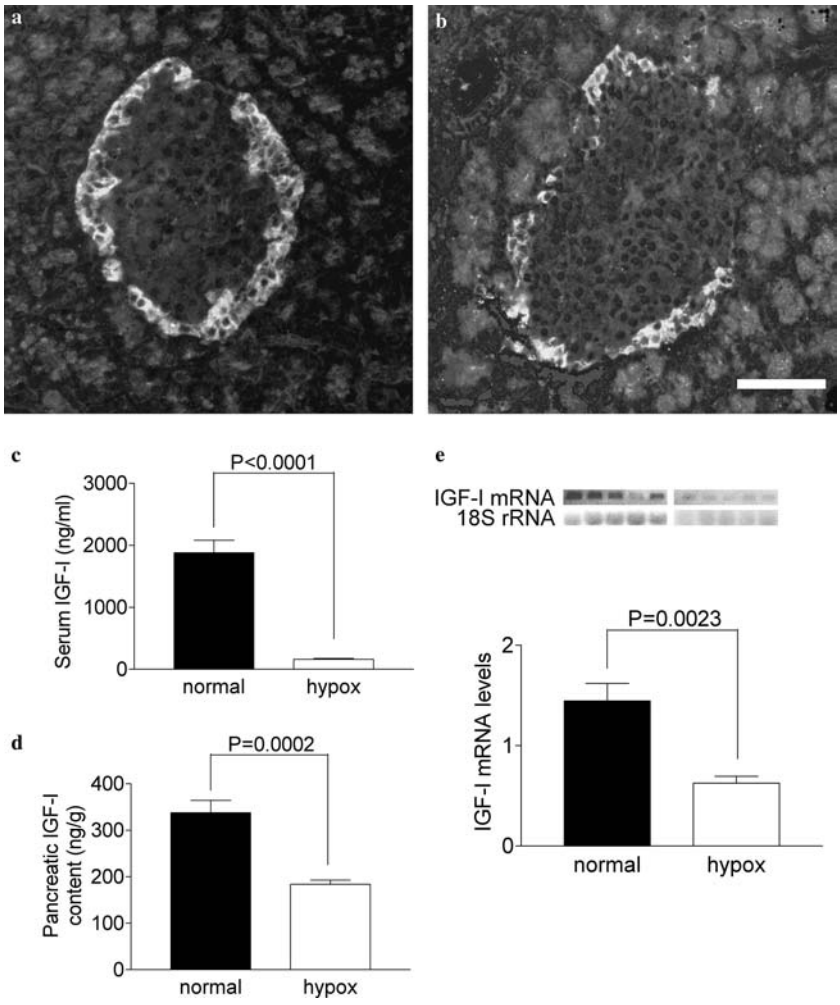

Fig. 4 Localization of IGF-I peptide in **a** normal and **b** hypophysectomized (hypox) rats. Bar: 60 µm. Serum IGF-I (**c**), pancreatic IGF peptide content (**d**), and IGF-I mRNA levels (**e**) of normal and hypox rats. Serum and pancreatic IGF-I were measured by RIA. IGF-I mRNA was determined by Northern blot. IGF-I mRNA and 18S rRNA signals are shown above the corresponding columns for each of two groups (**e**). IGF-I mRNA signals were normalized against the 18S rRNA signals and expressed as relative levels. *Columns* give the mean values ($n = 5$), and *bars* indicate SEM. Significance levels are shown in **c**–**e**

139 ± 22 ng per pancreas, respectively, $P = 0.0091$). This increase was accompanied by a 2.2-fold increase in somatostatin mRNA ($P < 0.0001$; Fig. 3f).

Serum IGF-I concentrations and pancreatic IGF-I peptide and mRNA levels

Hypophysectomy caused a 11.7-fold decrease in total serum IGF-I (161 ± 15 ng/ml vs. $1{,}881 \pm 201$ ng/ml in normal rats; $P < 0.0001$, Fig. 4c). In order to correct the pancreatic IGF-I content for the IGF-I present in the blood of tissue capillaries, we measured the blood fraction in the pancreas of normal rats. Normal pancreatic tissue contained $5.26 \pm 0.42\%$ blood (mean ± SEM, $n = 3$). For hypox rats, this value was corrected according to their smaller blood volume (87% of normal) (Gemzell and Sjostrand 1954). Based on a mean hematocrit of 46%, the mean serum volume in 1 g of normal pancreas was 28.40 ± 2.25 µl and ~24 µl in 1 g of hypox pancreas . Therefore, all IGF-I values obtained for pancreatic extracts were corrected for IGF-I present in the above serum volumes. The pancreatic IGF-I content in hypox rats was, therefore, 54% of that in the pancreas of normal animals (184 ± 9 vs. 338 ± 26 ng/g, respectively; $P = 0.0002$, Fig. 4d). When expressed per whole pancreas, the IGF-I content was 51.0 ± 2.8 ng per pancreas for hypox compared with 143 ± 8 for normal rats. The decrease in IGF-I in serum and pancreatic tissue after hypophysectomy was accompanied by a pronounced decrease in pancreatic IGF-I mRNA levels ($P = 0.0023$, Fig. 4e).

Discussion

In this study, we investigated the effect of hypophysectomy in rats on the pancreatic peptide and mRNA levels of the three major islet hormones insulin, glucagon, and somatostatin, and possibly related changes in serum glucose, insulin, and glucagon. Hypophysectomy was carried out in 5-week-old animals, which were used 2 weeks later. Beside growth failure, a dramatic fall in the serum IGF-I level reflected GH deficiency.

The drop in serum IGF-I was accompanied by a decrease in immunoreactive pancreatic IGF-I, as assessed by immunofluorescence, which localized IGF-I to

the α cells of the islets (Maake and Reinecke 1993; Jevdjovic et al. 2004), and by radioimmunological determination of IGF-I in pancreas extracts. Furthermore, a concomitant decrease in IGF-I mRNA was documented by Northern blot analysis. The decrease in pancreatic IGF-I peptide and mRNA was less dramatic than the decrease in serum IGF-I, indicating that GH deficiency primarily affects the liver, the main production site of IGF-I. Nevertheless, the specific localization of IGF-I in α cells, as well as the GH-dependent regulation of IGF-I peptide in these cells and of the pancreatic IGF-I mRNA (Jevdjovic et al. 2004), suggests that IGF-I is produced in the pancreatic islets. Another study has shown that IGF-I is also secreted from rat pancreatic islets (Swenne and Hill 1989).

The reduced serum insulin and elevated glucagon concentrations observed in our study agree with earlier findings in hypox rats (Van Lan et al. 1974). Despite decrease in serum insulin, serum glucose and pancreatic insulin content per gram of tissue were not significantly different from the normal animals, as also reported by Malaisse et al. (1968), and the insulin mRNA level was elevated, as demonstrated by ISH and Northern blot analysis. These findings might be interpreted as a reflection of reduced translational efficiency together with a blunted insulin response to glucose due to the lack of GH. Several studies have shown that the insulin response to glucose is delayed and/or reduced in hypox-rat-isolated islets (Parman 1975) or in hypox rats in vivo (Penhos et al. 1971; Heinze et al. 1981). In another study, the first and second phase of insulin secretion in the perfused pancreas of hypox rats have been found to be depressed (Curry and Bennett 1973).

In the hypox rats, 1.3-fold elevated glucagon content per gram of pancreas, as measured by RIA and qualitatively confirmed by IHC, was paralleled by a similar (1.6-fold) increase in serum glucagon whereas glucagon mRNA by both Northern blot analysis and ISH showed a more pronounced rise (4.5-fold by Northern blot analysis). When comparing the relative ratios between glucagon mRNA and peptide in normal (1:10) and hypox rats (1:3), it appears that the translational efficiency is reduced in the hypox animals. The corresponding ratios for insulin mRNA and peptide (1:110 in normal rats and 1:40 in hypox rats) also support this reasoning, as mentioned earlier.

GH regulates its own secretion by regulating the synthesis and secretion of somatostatin in the hypothalamus (Giustina and Veldhuis 1998). Thus, GH deficiency after hypophysectomy results in a decrease in the hypothalamic somatostatin content in vivo and its release in vitro (Berelowitz et al. 1981). In contrast, pancreatic somatostatin has been reported to be increased in rats 4–6 weeks after hypophysectomy (Recant et al. 1984). The latter result agrees with the results of the present study with respect to the pancreatic somatostatin peptide content. In addition, we also show that somatostatin mRNA, as assessed by two different methods, was significantly increased. Unfortunately, it was not possible to measure serum somatostatin because the serum volume required for this RIA (5 ml) by far exceeded the amount obtained from one rat. It appears from the above-mentioned relative ratios between somatostatin peptide and mRNA that the translational efficiency was increased rather than decreased in hypox (1:320) compared with normal (1:200) animals.

In summary, the results of our study show that the fall of serum IGF-I after hypophysectomy goes along with a concomitant decrease of pancreatic IGF-I peptide and mRNA but with partly discordant changes of the serum concentrations of insulin and glucagon and the islet peptide and/or mRNA content of the three major islet hormones. It appears that GH deficiency resulting in a "low IGF-I state" affects translational efficiency of these hormones as well as their secretory responses. Finally, the maintenance of normoglycemia in the presence of reduced serum insulin and elevated serum glucagon concentrations, both of which would be expected to raise the blood sugar level, may result mainly from enhanced insulin sensitivity of insulin target tissues, possibly due to GH deficiency and the subsequent decrease of IGF-I production (Yakar et al. 2004).

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