Elevated Blood Pressure and Enhanced Myocardial Contractility in Mice with Severe IGF-1 Deficiency

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Abstract

To circumvent the embryonic lethality of a complete deficiency in insulin-like growth factor 1 (IGF-1), we generated mice homozygous for a site-specific insertional event that created a mutant IGF-1 allele ($igf1^m$). These mice have IGF-1 levels 30% of wild type yet survive to adulthood, thereby allowing physiological analysis of the phenotype. Miniaturized catheterization technology revealed elevated conscious blood pressure in IGF-$1^{m/m}$ mice, and measurements of left ventricular contractility were increased. Adenylyl cyclase activity was enhanced in IGF-$1^{m/m}$ hearts, without an increase in β-adrenergic receptor density, suggesting that crosstalk between IGF-1 and β-adrenergic signaling pathways may mediate the increased contractility. The hypertrophic response of the left ventricular myocardium in response to aortic constriction, however, was preserved in IGF-$1^{m/m}$ mice. We conclude that chronic alterations in IGF-1 levels can selectively modulate blood pressure and left ventricular function, while not affecting adaptive myocardial hypertrophy in vivo. (*J. Clin. Invest.* 1996. 98:2648–2655.) Key words: insulin-like growth factor I • mutagenesis, site-directed • myocardial contraction • hypertension • adrenergic receptors • β receptors

Introduction

Insulin-like growth factor 1 (IGF-1) is a pleiotropic growth signal produced by numerous tissues in response to growth hormone (GH)[1] and local stimuli. In addition to its effects on tissue growth, IGF-1 also influences function. Recently, we (1) and others (2, 3) reported the generation of mice (IGF-$1^{-/-}$) with a complete disruption of the *igf1* gene. Our heterozygous mice (IGF-$1^{+/-}$) exhibit decreased serum IGF-1 levels and are somewhat smaller than wild type (IGF-$1^{+/+}$) littermates; however, the size reduction was proportional in most organs and all tissues appeared histologically normal. IGF-$1^{+/-}$ mice were fertile; however, when bred to homozygosity, more than 95% of the IGF-$1^{-/-}$ pups died at birth. Dead neonates were just over half the size of their littermates and did not breathe.

A number of experimental studies have suggested a potential role of both GH and IGF-1 in cardiovascular physiology and function. However, the physiological role of IGF-1 in cardiovascular regulation is unclear, as no pharmacological inhibitors of IGF-1 are available. The vasoactive effects of IGF-1 following in vivo administration suggest the possibility that IGF-1 may have a role in the control of blood pressure (4, 5), while the increase in mRNA for IGF-1 and its receptor in hearts subjected to pressure overload (6–8) implicate IGF-1 in adaptive cardiac growth. Recently, it has been shown that IGF-1 administration after experimental myocardial infarction in the rat produces hypertrophy of the noninfarcted myocytes with increased ventricular function in animals with large infarcts (9); ventricular contractility is enhanced when either GH or IGF-1 is administered to normal rats (10), and a preliminary study of GH (sufficient to double IGF-1 levels) in humans with dilated cardiomyopathy demonstrated improved wall stress, hemodynamics, myocardial energy metabolism, and clinical status (11). An animal model of IGF-1 deficiency, coupled with miniaturized catheterization technology to assess in vivo cardiovascular physiological phenotypes (12–15), would provide an appropriate vehicle to assess the need for IGF-1 in normal cardiovascular regulation. We describe a viable strain of mice with severe IGF-1 deficiency, who demonstrate chronically elevated blood pressure, enhanced cardiac contractility, and a preserved left ventricular hypertrophic response to pressure overload.

Methods

RNA isolation and RT-PCR. PolyA+ mRNA was isolated from 100 mg of each wild type and midi tissue using a mRNA STAT kit (TEL-TEST B, Inc., Friendswood, TX). cDNA was primed using an oligonucleotide homologous to exon 4 {I-CDNA 102: 5′-CTG AGT CTT GGG CAT GTC AGT GTG G-3′} (Perkin-Elmer Cetus Instruments, Emeryville, CA). PCR was carried out for 35 cycles (94°C for 30 s, 65°C for 30 s, 74°C for 2 min), using I-CDNA 102 as the antisense primer and an oligonucleotide specific for the 3′ end of exon 3 {I-CDNA 106: 5′-CGT GTG TGG ACC GAG GGG CTT TTA C-3′} as the sense primer. PCR products were separated on a 0.8% agarose gel, and transferred to a nylon membrane (Zetaprobe; Bio-Rad, Richmond, CA) using standard techniques. Blots were probed with ^{32}P-end labeled oligonucleotides, specific for exon 4 {5′ to I-CDNA 102 sequence above, I-CDNA 103: 5′-GAC AGG CAT TGT GGA TGA GTG TTG C-3′} or specific for the 5′ end of exon 3 {I-CDNA 104: 5′-CAC AGC TGG ACC AGA GAC CCT TTG-3′}. Prehybrid-

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1. *Abbreviations used in this paper:* GH, growth hormone; LV, left ventricle; LV dP/dt, left ventricle pressure; TAC, transverse aortic constriction.

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ization and hybridization were performed in 3 × SSC, 20 mM NaH_2PO_4, pH 7.0, 7% SDS, 10 × Denhardt's with 100 μg salmon sperm DNA at 50°C. The midi cDNA was not detectable by PCR when total RNA was used for RT; possibly, the presence of the extra 1.8 kb of neomycin cassette contributes to mRNA instability. The PCR reaction under the conditions used is not quantitative and was not designed to be representative of mRNA levels either between tissues or animals.

Measurements of pituitary GH levels. Frozen pituitaries were homogenized in 0.5 ml lysis buffer (0.3 M sucrose, 10 mM Tris pH 8.0, 3 mM $CaCl_2$, 2 mM $MgCl_2$, 0.15% Triton X-100, 0.5 mM DTT in DEPC-treated H_2O) and layered over 0.25 ml cushion buffer (same as lysis buffer, except 0.4 M sucrose) in microcentrifuge tubes. Samples were centrifuged at 2,200 rpm for 10 min at 4° C, and the upper layer transferred to clean tubes. 0.1 ml of diluted homogenate was added to a well of an immunoplate (Nunc Inc., Naperville, IL) which had been precoated with 0.1 ml of 1:697 goat anti–rat antibody (lot 19898–54; Genentech Inc., South San Francisco, CA) for 15 h at 4°C and blocked with 0.5% BSA. Samples were added in duplicate, along with controls and reference standard (NIH rat GH RP-2, AFP-3190 B), and incubated at room temperature for 2 h. Plates were then washed six times with PBS containing 0.05% Polysorbate 20 and incubated with 0.1 ml of 1:10 sheep anti–rat GH-HRP (Genentech lot 19898–15) at room temperature for 2 h, then washed as before. 0.1 ml of HRP substrate (2 component TMB Laboratories, Inc., Gaithersburg, MD; Kirkegaard & Perry) was added to each well and plates incubated 25 min. Reaction was stopped by adding 0.1 ml 1M H_3PO_4 and ODs read on a plate reader at 450–650 nm.

Measurements of hormone levels. Mice were fasted overnight and blood collected retroorbitally. Serum was assayed for total IGF-1 levels by radioimmunoassay (16): to 50 μl serum, 0.7 ml cold acid/ethanol (12.5% 2 N HCl, 87.5% EtOH) was added to separate binding proteins from IGF-1. After a 30-min incubation on ice, samples were spun 5 min in a microcentrifuge at 10,000 rpm, and 0.15 ml of the supernatant was neutralized with 0.03 ml 1 M Tris base. 0.1 ml of the neutralized supernatant was added to 0.4 ml assay buffer (1 × PBS, 0.1% gelatin, 0.05% Polysorbate 20, 0.01% thiomerosal) and assayed for IGF-1 by RIA utilizing ^{125}I–IGF-1 (20,000 cpm), rabbit anti–IGF-1 antibody, and goat anti–rabbit Immunobeads (BioRad International, Camarillo, CA). Insulin levels were measured using a RIA kit (Linco Research, St. Louis, MO), serum GH levels by immunoassay as described by Elias et al. (17), and glucose measured using a One Touch II monitor (Lifescan, Milpitas, CA).

Conscious blood pressure measurement. Mice were housed up to four per cage in a room in which temperature was controlled (20±1°C) and maintained on a 12-h light–dark cycle. Under anesthesia (ketamine 100 mg/kg i.p. plus xylazine 5 mg/kg i.p.), the femoral artery was isolated at the ventral junction of the right hindlimb with the abdomen. A flame-stretched PE50 catheter was inserted into the vessel through an arteriotomy and advanced into the abdominal aorta. The catheter containing heparinized 0.9% saline was securely sutured in place, plugged, and tunneled under the skin to exit through a small incision at the dorsal aspect of the neck. After recovery from anesthesia, animals were moved to individual cages. After 2 d recovery, mice were placed into a quiet environment and the catheter connected to a P23 Db strain gage transducer (Stratham Instruments, Hato Rey, PR). The signal was passed to a pressure processor for pulsatile blood pressure and electronically determined mean arterial pressure, and then to a biotach for beat-to-beat heart rate determination (Gould Inc., Cleveland, OH). From the analogue tracings systolic and diastolic pressures were determined as well as computed mean arterial pressure, which was compared to the electronic mean and found to be indistinguishable. Measurements were obtained under conscious and unrestrained conditions, between 9 a.m. and 1 p.m., after an acclimation period of at least 30 min.

Left ventricular hemodynamics. Anesthetized, open chest left ventricular (LV) hemodynamics were performed as described (18, 19). Animals were anesthetized with a mixture of ketamine 100 mg/kg i.p. and xylazine 5 mg/kg i.p., the trachea was intubated, and the animal connected to a volume-cycled ventilator. A carotid artery was cannulated with a flame-stretched PE 50 catheter connected to a modified P50 Statham transducer. The chest and pericardium were then opened and a 2F high-fidelity micromanometer catheter (Millar Instruments, Inc., Houston, TX) was inserted through the left atrium advanced across the mitral valve, and secured in the LV inflow tract. Hemodynamic measurements were recorded at baseline and 45 to 60 s after injection of graded bolus doses of isoproterenol. Continuous aortic pressure, LV systolic and diastolic pressures, and the first derivative of LV pressure (LV dP/dt) were recorded on an eight-channel chart recorder and in digitized form on computer disk for beat averaging. 10 sequential beats were averaged for each measurement. The dose range of isoproterenol was previously determined to have an inotropic effect with minimal effect on HR (18, 19).

Acute and chronic aortic constriction. In separate experiments mice were instrumented as previously described (12, 13), and an additional thoracotomy was performed in the second intercostal space. The transverse aorta between right and left carotid arteries was isolated and a 7-0 nylon suture ligature was placed around the aorta, with the two ends of the suture left outside the chest. Hemodynamic recording was continuously acquired under basal conditions and during a 5 s acute transverse aortic occlusion produced by gently pulling on the suture ends. To study the effect of a transient increase of aortic and LV pressure on hemodynamic parameters, one beat just before occlusion and three beats after the occlusion were chosen for analysis. For studies of chronic transverse aortic constriction, the suture ligature was tied against either a 27 guage (wild type) or 27.5 gauge (IGF-$1^{m/m}$) needle, which was promptly removed to result in a significant pressure load on the LV. The chest was closed, mice were allowed to recover, and 7 d after transverse aortic constriction (TAC) simultaneous left and right carotid pressures were measured under anesthesia to assess the degree of pressure load. Immediately following, the hearts were removed and chambers dissected and weighed.

Adenylyl cyclase activity and β-adrenergic receptor binding density. Ventricles were homogenized in 5 ml of cold lysis buffer (5 mM Tris Cl, 5 mM EDTA) and spun at 500 g × 10 min to remove nuclei and cellular debris (18–20). The supernatant was passed over cheese cloth and membranes pelleted by centrifugation at 40,000 g × 15 min. Membranes were resuspended in 75 mM Tris Cl, 12.5 mM $MgCl_2$, and 4 mM EDTA at concentration of 1 mg membrane protein/ml. 30 μg of membrane was incubated for 10 min at 37°C in 50 μl of assay mixture containing 20 mM Tris Cl, 0.8 mM $MgCl_2$, 2 mM EDTA, 0.12 mM ATP, 0.05 mM GTP, 0.1 mM cAMP, 2.7 mM phosphoenolpyruvate, 0.05 IU/ml myokinase, 0.01 IU/ml pyruvate kinase, and [α-^{32}P]cAMP was isolated and cAMP quantitated (18).

Saturation ligand binding assays were performed on crude membrane preparations in 500 μl of binding buffer. Each reaction contained saturating quantities of the radioligand ^{125}I-cyanopindolol. Nonspecific binding was determined in the presence of 20 μM alprenolol. Binding assays were conducted at 37°C for 60 min and terminated by rapid vacuum filtration over glass fiber filters, which were subsequently washed and counted in a gamma counter. Specific binding was normalized to membrane protein and reported as mean femtomoles of receptor/mg (18–20).

Results

Site-specific insertion generates viable homozygotes. During the generation of the IGF-1 null mice described previously (1) a single AB.1 embryonic stem (ES) cell clone was obtained in which there had been a site-specific insertion of the targeting construct instead of an homologous recombination (Fig. 1). Site-specific insertion of the construct was possible because the construct was linearized at the junction of the vector sequences with *igf1* intron sequences and, although the construct contained thymidine kinase gene sequences, negative selection

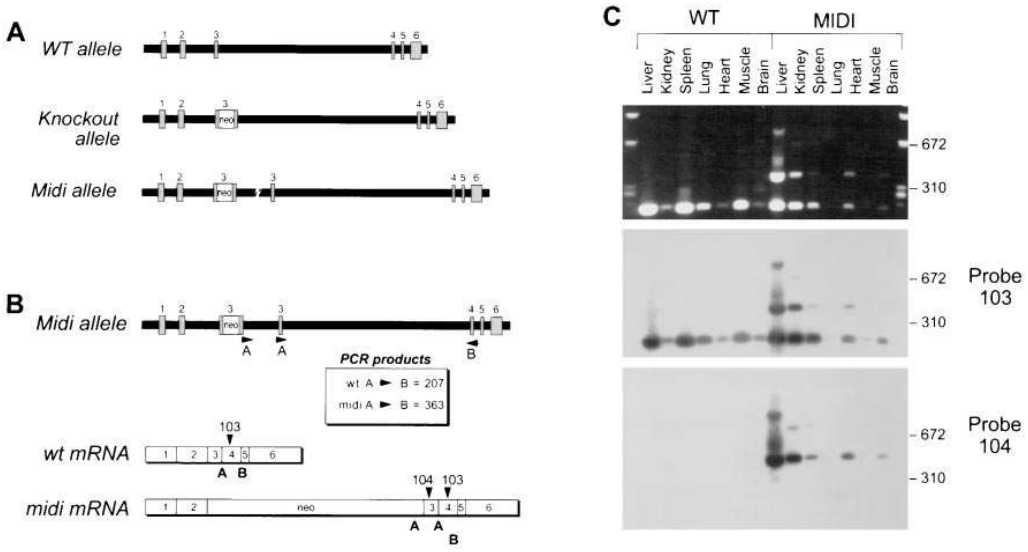

Figure 1. (*A*) Diagrammatic representation of the 5′ end of wild type and targeted murine *igf1* alleles showing the gene structure of the full null (−) and the site-specifically inserted midi (m) alleles. (*B*) Diagram of expected PCR products from the wild type and midi mRNAs; primer A is oligonucleotide I-CDNA 106, specific for the 3′ end of exon 3, primer B is oligonucleotide I-CDNA 102, specific for sequences in exon 4. (*C*) Panel 1, RT-PCR products from various wild type and homozygous IGF-1$^{m/m}$ tissues, amplified using primers A and B above. The 363 bp band is accounted for by the presence of additional exon 3 sequences in the midi mRNA, not present in wild type tissues. Panel 2, Southern blot of the PCR products from Panel 1, probed with an oligonucleotide specific for exon 4 (I-CDNA 103), identifying both wild type and midi cDNAs. Panel 3, same blot probed with an oligonucleotide specific for the 5′ end of exon 3 (I-CDNA 104); this sequence is upstream of I-CDNA 106, and thus is not present in the wild-type 207 bp PCR product, but detects the duplicated exon 3 sequences in the 363 bp midi PCR product.

was not used during ES cell clone growth (21). The *igf1* allele in this clone contained the entire construct including the neomycin^r interrupted exon 3, the thymidine kinase gene, and vector sequences inserted 5′ of the endogenous exon 3. The genomic organization of the site-specific insertion allele was confirmed by diagnostic restriction digestion of DNA (BglII, KpnI, SacI, and BamHI each give unique fragments not seen in either the wild type or the null alleles), with southern blotting using probes from the targeting sequences (data not shown). Mice heterozygous for this allele exhibited reduced IGF-1 serum levels and body weights similar to those seen for mice heterozygous for the null allele. However, in contrast to the *igf1* null mice, mice homozygous for this mutant gene were viable and fertile but profoundly growth retarded, being intermediate in size between wild type and IGF-1$^{-/-}$ (Table I); because of their intermediate size, they have been identified as "midi" mice (IGF-1$^{m/m}$), and had low but detectable IGF-1 in their sera. If the mutant allele *igf1^m* did not exhibit alternative splicing, this would result in a null allele and no IGF-1 protein could be produced, as there are multiple stop codons in all three reading frames in the neomycin^r cassette, which is inserted in an antisense orientation 13 amino acids after the start of the mature protein in exon 3. However, alternative splicing is known to occur with exon 5 in the wild type rodent *igf1* gene, and we postulated that alternative splicing was occurring around the neomycin^r-containing exon, resulting in the production of a small amount of wild type mRNA and protein. Exhaustive rtPCR was used to establish that wild type mRNA for IGF-1 was present in the IGF-1$^{m/m}$ mice (Fig. 1, *B* and *C*). We were also able to detect the mutant mRNA in this manner.

After injection of recombinant clone cells into C57BL/6J blastocysts, three founder chimeras were mated with C57BL/6J females to produce F1 heterozygous animals (129/SvX C57BL/6J). These animals were intercrossed to generate F2 homozygous, heterozygous, and wild type mice. Mating of the F2 homozygous animals to each other to produce F3 homozygote animals, and parallel mating of sibling F2 wild type animals to each other to produce F3 wild type controls generated the animals used in this study. Large numbers of F2 animals were used to generate animals with as random a genetic background as possible, and to avoid the possibility of genetic founder events.

Severely IGF-1 deficient young adult mice have reduced serum glucose. As seen in Table I, serum IGF-1 levels in IGF-1$^{m/m}$ mice were 30% of wild type values. Plasma insulin levels appeared to be slightly higher in these young adult IGF-1$^{m/m}$ mice than in age-matched controls, although this difference did not reach statistical significance. Serum glucose was significantly lower in the IGF-1$^{m/m}$ mice than in age-matched controls, however; insulin resistance, as determined by glucose tolerance testing, was not present at this age (data not shown). Pituitary growth hormone content was significantly lower in IGF-1$^{m/m}$ mice than in wild type; although random serum growth hormone levels do not accurately reflect the pulsatile secretion of GH, there was a trend for higher values in IGF-1$^{m/m}$ mice compared with controls (Table I), suggesting that the reduced pituitary GH content is not indicative of decreased synthesis.

Cardiac chamber weights are reduced in proportion to body weight. The body weights of adult IGF-1$^{m/m}$ mice were significantly reduced by 36% compared with their wild type counterparts (Table I), comparably in males and females. Spleen and thymus were reduced proportionately to body weight, while brain, kidneys, and liver were less affected (L. Powell-Brayton, unpublished observations). Heart weight in adult IGF-1$^{m/m}$ mice was likewise reduced, by 31.0% vs. wild type, due to proportionate decreases in all chambers. Tibial length was decreased to a lesser extent in IGF-1$^{m/m}$ mice (10.6%, Table I); in our experience, tibial length correlates highly with body weight in normal young adult mice and shows much less variability after maturity (H. Rockman and J. Hunter, unpublished observations), similar to previous results in adult rats (22). Thus, in attempting to normalize LV mass for body size, when

Table I. Principal Characteristics of IGF-1m/m Mice and Age-matched Wild Type Controls

	Wild type	IGF-$1^{m/m}$	% Change from WT	*P*
Body weight (g)	32.32±1.0 ($n = 16$)	20.75±1.12 ($n = 16$)	−36%	< 0.0001
Tibial length (mm)	18.80±0.21 ($n = 16$)	16.80±0.11 ($n = 16$)	−11%	< 0.0001
LV mass (mg)	111.3±4.9 ($n = 16$)	77.8±4.0 ($n = 16$)	−30%	0.00001
Serum IGF-1 (ng/ml)	164.841±11.596 ($n = 24$)	51.248±8.514 ($n = 26$)	−69%	< 0.001
Serum GH (ng/ml)	1.604±0.546 ($n = 12$)	7.616±3.781 ($n = 17$)	+375%	0.20
Plasma insulin (ng/ml)	0.493±0.228 ($n = 7$)	0.948±0.289 ($n = 6$)	+92%	0.24
Serum glucose (mg/dl)	87.857±5.002 ($n = 7$)	45.833±6.462 ($n = 6$)	−48%	0.0003
Pituitary GH content (mg/pit)	48.603±8.72 ($n = 18$)	24.826±4.501 ($n = 22$)	−49%	0.02

Values are mean±SE.

one uses body weight IGF-$1^{m/m}$ LVs are larger (3.77±0.09 mg/g vs. wild type 3.43±0.09 mg/g, $P = 0.01$), while using tibial length IGF-$1^{m/m}$ LVs are smaller (4.62±0.22 mg/mm vs. wild type 5.95±0.29 mg/mm, $P = 0.001$); therefore there is no consistent difference between chamber weights in severely IGF-1 deficient mice and controls beyond that explained by body size alone. There were no gross histological abnormalities in IGF-$1^{+/m}$ or IGF-$1^{m/m}$ animals.

Severely IGF-1–deficient mice have elevated arterial blood pressure. Direct arterial blood pressure was measured in conscious, unrestrained animals using an indwelling femoral artery catheter no sooner than 48 h after catheter implantation. Heart rate in IGF-$1^{m/m}$ mice (3–4 mo of age) was not significantly different from that in control animals (625±16 bpm [$n = 11$] vs. 597±28 bpm [$n = 9$]). However, there was a significant elevation of mean arterial pressure in the severely IGF-1 deficient IGF-$1^{m/m}$ mice compared with wild-type controls (125±1 mmHg vs. 112±1 mmHg, $P < 0.001$; Fig. 2). Diastolic pressure also was higher (100±3 vs. 91±3 mmHg, $P < 0.05$) in IGF-$1^{m/m}$ mice, but the increase in systolic (146±4 vs. 135±4 mmHg, $P = 0.07$) pressure attained only borderline statistical significance. Heterozygote animals had no detectable differences in any parameter (data not shown). In separate experiments, conscious blood pressures in a small group ($n = 3$) of IGF-$1^{-/-}$ (null) homozygotes (1), which survive to a limited extent in a different genetic background (CD-1), were compared to those in age and sex-matched IGF-$1^{+/+}$ littermates ($n = 4$). Mean arterial pressure in IGF-$1^{-/-}$ was higher than in wild type (122±12 mmHg vs. 107±1 mmHg), but this did not reach statistical significance due to the small sample size. Thus, despite homeostatic mechanisms involved in blood pressure control, the arterial pressure was higher in IGF-$1^{m/m}$ mice than in wild type animals, clearly suggesting a role of IGF-1–dependent pathways in the control of basal blood pressure; the trend toward higher blood pressure in IGF-1 null mice in the CD-1 background confirms a specific effect of IGF-1 deficiency on blood pressure across different genetic substrates.

Cardiac contractility is increased in IGF-$1^{m/m}$ mice. The importance of growth hormone in the regulation of myocardial contractile force has recently been demonstrated in GH-deficient dwarf rats (23), raising the possibility that IGF-1, a prominent endocrine mediator of GH effects, may be involved in cardiac contractility. To examine whether IGF-1 signaling pathways are necessary for the maintenance of normal cardiac function, we measured both basal and isoproterenol-stimulated cardiac contractility in IGF-1 deficient mice and wild type controls. Cardiac catheterization and in vivo hemodynamic measurements were performed in anesthetized mice (13) with continuous recording of heart rate (HR), aortic pressure, LV dP/dt, and the first derivative of LV dP/dt at baseline and after progressive doses of isoproterenol (18). Before thoracotomy, mean aortic pressure was significantly increased in anesthetized IGF-$1^{m/m}$ mice compared to wild type (81±3 mmHg [$n = 4$] vs. 71±3 [$n = 4$] mmHg, $P < 0.05$), confirming that differences obtained in conscious animals remain present under anesthesia. After thoracotomy, basal LV systolic pressure was similarly elevated in IGF-$1^{m/m}$ mice (87.0±5.9 mmHg [$n = 12$] vs. 70.2±1.4 mmHg [$n = 13$], $P < 0.0001$) (Fig. 3 *A*). The heart rate was slightly but significantly higher in IGF-$1^{m/m}$ mice at control and with lower doses of isoproterenol, but it was identical to that in wild type animals at high doses (Fig. 3 *B*). Although a trend toward higher LV end-diastolic pressure was present in the IGF-$1^{m/m}$ group compared with wild type, this difference did not reach statistical significance. Surprisingly, the maximum first derivative of the LV pressure (LV dP/dt_{max}), an index of myocardial inotropic state, was significantly increased in the IGF-$1^{m/m}$ mice compared to wild type controls (6395±684 mmHg/s vs. 4023±277 mmHg/s, respectively, $P < 0.0001$, Fig. 3 *C*). Similarly, LV dP/dt_{min}, an index of relaxation, was also enhanced in the IGF-1 deficient mice (−4840±648 mmHg/s vs. −3123±212 mmHg/s, $P < 0.0008$, Fig. 3 *D*); none of these differences was seen in heterozygote animals. These data indicate that a severe in vivo deficiency of IGF-1 is associated with a significant increase in LV inotropic state.

LV dP/dt_{max} has previously been demonstrated to be dependent on preload but relatively independent of isolated

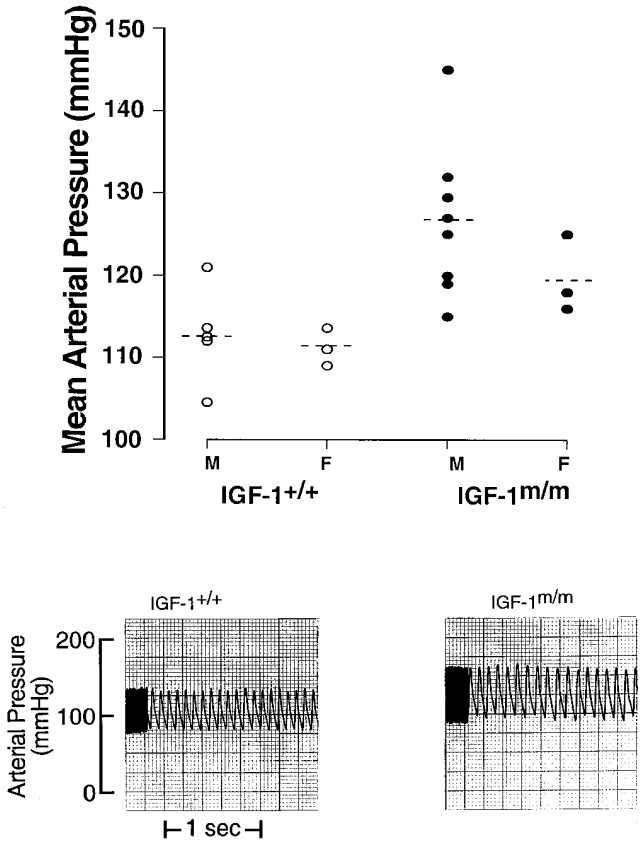

Figure 2. (*Top*) Individual measurements of mean arterial pressure in male and female IGF-$1^{m/m}$ mice and their wild type controls. $^{*}P < 0.01$, and $^{\ddagger}P < 0.05$, compared to sex-matched wild type, Student's *t* test. (*Bottom*) Examples of recordings of pulsatile arterial pressure in IGF-$1^{m/m}$ and wild type animals.

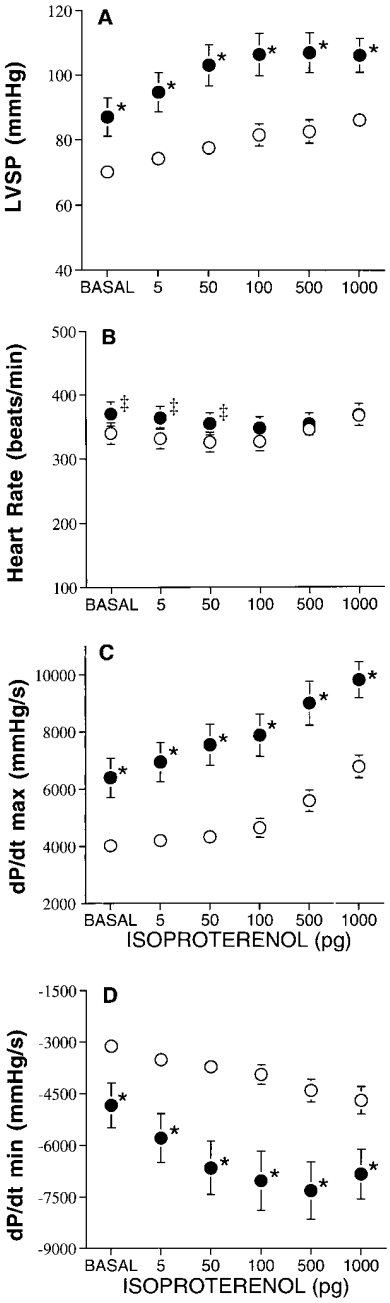

Figure 3. Hemodynamic variables are shown at baseline and after graded doses of isoproterenol. (*A*) peak LV systolic pressure, (*B*) heart rate LV end-diastolic pressure, (*C*) LV dP/dt_{max} (*D*) LV dP/dt_{min}. Data are reported as mean±SEM. Open and filled circles represent wild type (n = 13, 6 male and 7 female) and IGF-$1^{m/m}$ mice (n = 12, 5 male and 7 female), respectively. Statistical analysis was performed using repeated measures analysis of variance with post hoc (Scheffe's) analysis. $^{*}P < 0.001$ vs. wild type; $^{\ddagger}P < 0.05$ vs. wild type. Differences were all significant for both males and females individually, and the genders were therefore combined.

changes in the LV systolic pressure except when a low aortic diastolic pressure prevents the development of LV dP/dt_{max} (24). To exclude the possibility that low diastolic pressure was a factor in the wild type mice and that enhanced LV dP/dt_{max} was secondary to a hemodynamic effect of increased arterial pressure in IGF-1 deficient mice, we measured LV dP/dt_{max} under conditions of acutely increased LV systolic pressure induced by thoracic aortic constriction in wild type mice. As shown in Fig. 4, in initial beats at pressures encompassing the range of LV pressure previously observed in IGF-$1^{m/m}$ mice, no difference in LV dP/dt_{max} was recorded. Thus, the enhanced contractility in the IGF-$1^{m/m}$ animals does not appear to be secondary to the increase in blood pressure per se.

To exclude an increase in β-adrenergic receptor density as the etiology, binding assays demonstrated that the cardiac β receptor density was comparable between IGF-$1^{m/m}$ mice and wild type (30.14±3.15 vs. 31.04±0.83 fmol/mg protein, P = NS, n = 5 in each group). However, basal adenylyl cyclase activity was enhanced in the IGF-$1^{m/m}$ mice compared to controls (9.16± 1.61 [n = 10] vs. 4.88±0.69 [n = 7] pmol cAMP/mg protein per min, respectively, P = 0.05). In the absence of an appreciable change under basal conditions in two primary regulators of LV performance that are sensitive to catecholamine levels, i.e., the heart rate and the LV end-diastolic pressure, it is suggested that enhanced coupling of β-adrenergic receptors to downstream signaling pathways rather than increased circulating catecholamines may contribute to the increased myocardial contractility reflected in augmented LV dP/dt_{max}. These results are in contrast to those obtained in a genetic rat model of GH deficiency, the primary regulator of IGF-1 biosynthesis, in which increased basal cardiac contractility has been reported in the setting of increased β-adrenergic receptor density (23).

Hypertrophic response to hemodynamic load is preserved in severe IGF-1 deficiency. IGF-1 has been shown to induce hypertrophy of neonatal rat cardiac myocytes in vitro (25) and in the normal rat left ventricle and the infarcted heart in vivo (9); others have not found a significant hypertrophic effect of IGF-1 on ventricular myocytes in vitro, however (26). mRNA for both IGF-1 and its receptor are upregulated in the ventricle of animals subjected to pressure overload (6–8) or infarction (27), raising the possibility that IGF-1 may be an important autocrine/paracrine mediator of adaptive cardiac hypertrophy. To determine whether normal levels of IGF-1 are required for the hypertrophic response to pressure overload, severely IGF-1 deficient IGF-$1^{m/m}$ mice and nontransgenic controls underwent transverse aortic constriction or sham operation using previously established techniques (13). As shown in Fig. 5, when normalized for either body weight or tibial length, LV mass in the IGF-$1^{m/m}$ mice increased to a comparable extent (+47% vs. +48%, normalized for body weight) to wild type animals; the range of transstenotic pressure gradients was comparable between the two groups. Thus, the adaptive hypertrophic response of the left ventricle is unimpaired in the IGF-$1^{m/m}$ mice, documenting selectivity of the effects of IGF-1 deficiency on contractility and resting blood pressure.

Discussion

The identification of a line of mice harboring a site-specific insertion in the IGF-1 gene has allowed us to study the effects in adult animals of severe IGF-1 deficiency, a condition in which complete absence of IGF-1 is lethal at birth. At present, no pharmacological inhibitors specific for IGF-1 signaling are available, so this model is the first to provide insights into the in vivo physiological role of IGF-1 on basal cardiovascular function. These animals exhibit a functional phenotype which is distinct from that seen in GH deficient dwarf rats, in which both GH and IGF-1 are reduced.

Blood pressure. Essential hypertension is a polygenic disorder that is a major contributor to morbidity and mortality in human cardiovascular disease. Recent advances have led to the identification of a large number of candidate genes which might regulate basal blood pressure; however, to date, the genes which have demonstrated linkage with hypertension are those in the renin–angiotensin pathway (28) and in adrenal steroid biosynthesis (29). Mice transgenic for additional copies of the angiotensinogen gene have a gene-dosage dependent increase in blood pressure (30), while genetic deficiency of the vasoactive peptide ANP, generated by targeted disruption of the gene, has produced a murine model of salt-sensitive hypertension (31). It has been noted in this gene-targeted model that the number of copies of the Ren gene (two per haploid genotype inherited from the 129/Sv strain, one per haploid genotype inherited from the C57BL/6J strain) directly affects the blood pressure in individual animals (32). Our observations likewise suggest a strong influence of genetic modifiers on blood pressure. In IGF-$1^{m/m}$ animals in a CD-1 background (with reduction in IGF-1 levels comparable to that seen in 129/Sv × C57BL/6J) there is little difference in blood pressures from wild type (data not shown); in that background, however, IGF-1 null animals can survive, and in such mice (with no detectable IGF-1) there seems to be an increase in blood pressure comparable to that in IGF-$1^{m/m}$ mice in the 129/Sv × C57BL/6J background. These data indicate that the sensitivity of blood pressure to serum IGF-1 levels varies depending on the genetic background.

Igf1 could therefore be considered a candidate gene for blood pressure regulation, since it (like angiotensin II) has both growth-promoting properties for vascular smooth muscle

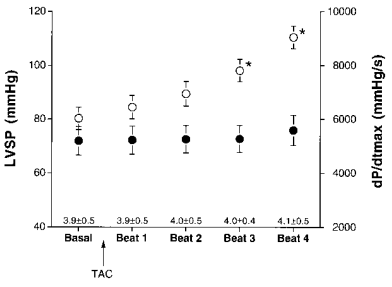

Figure 4. LV systolic pressure (*open circles*) and LV dP/dt_{max} (*filled circles*) before and for four beats after the creation of acute pressure overload with sudden transverse aortic constriction (TAC) in control (wild type) mice ($n = 10$). Data are reported as mean±SEM and include both male and female mice. Statistical analysis was performed using repeated measures analysis of variance with post hoc (Scheffe's) analysis. *$P < 0.0001$ vs. basal (before acute TAC). Numbers represent LVEDP.

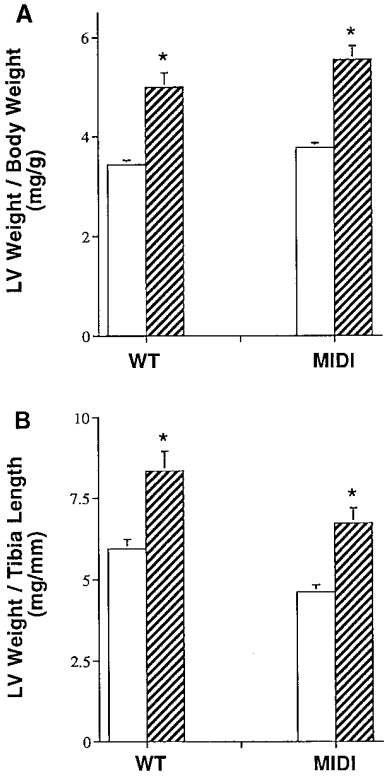

Figure 5. Response of IGF-$1^{m/m}$ mice and wild type controls to transverse aortic constriction (TAC). (*A*) LV weight normalized for body weight; (*B*) LV weight normalized for tibia length. Open bars denote sham-operated animals (wild type, $n = 16$; IGF-$1^{m/m}$, $n = 16$), hatched bars denote TAC (wild type, $n = 15$; IGF-$1^{m/m}$, $n = 14$). WT, wild type. Data are reported as mean±SEM and include both male and female mice. *$P < 0.01$ TAC vs. sham, *t* test.

cells (33) as well as potent vasodilator effects when administered in vivo (4, 5), suggesting a possible role for IGF-1 in the physiological regulation of vascular tone. IGF-1 produces vascular effects qualitatively similar to insulin, although at tenfold lower doses, indicating that the hemodynamic actions of insulin may be mediated through the IGF-1 receptor (34). In light of recent evidence that insulin and IGF-1 converge on a common signaling pathway (35), it is intriguing to note that resistance to the vasorelaxant effects of insulin, which has been reported in several pathological circumstances (including obesity (36), noninsulin dependent diabetes mellitus (37), and essential hypertension (38)), has been hypothesized to be a critical determinant of the increased blood pressure existing in each of these disease states (39). The increase in mean arterial pressure in IGF-$1^{m/m}$ transgenic mice, with parallel elevations of systolic and diastolic pressures, suggests a role for IGF-1 in maintaining the set point of arterial tone and vascular resistance. This finding is consonant with previous data on the vasodilator effect of IGF-1 infusion (4, 40, 41) which suggest that the effect on arterial pressure is at the level of the arteriolar resistance vessels, rather than an indirect effect on autonomic tone (the latter is also less likely, since no significant difference was seen in heart rate in conscious midi mice vs. wild type). Modulatory effects on sympathetic tone, as well as the possibility of an increase in cardiac output, cannot be excluded as contributory factors, however. The observation of enhanced adenylyl cyclase activity in cardiac myocyte extracts cannot readily be extrapolated as a mechanism for increased blood pressure; indeed, if also present in vascular smooth muscle one might anticipate a vasorelaxant effect from this pathway. Other potential contributors to the increase in blood pressure include plasma insulin and growth hormone (although transgenic mice overexpressing GH (42) and rats receiving GH over 5 wk (43) have no elevation in blood pressure; increases in renal plasma flow in response to GH may in fact be mediated by IGF-1 (44) GH deficient rats have lower mean arterial pressure than control Lewis rats (23) although recorded pressures in both groups are higher (132 ± 7 vs. 163 ± 8 mmHg, respectively) than in other rat strains (41). No relation has been found between body weight and mean arterial pressure in mice (H. Rockman, unpublished observations), so the lower body weight in the midi mice is unlikely to play a substantial role. The observation that IGF-1 deficiency produces elevated arterial pressure, regardless of mechanism, strongly suggests that IGF-1 signaling pathways contribute to blood pressure regulation, and that components of this pathway (IGF-1, its receptor, and the intracellular effector molecules under its control) are candidate genes for hypertension.

Left ventricular contractility. The finding of increased cardiac contractility was rather surprising, since our hypothesis had been that IGF-1 was essential for normal cardiac function. That this was not simply due to increased afterload due to increased arterial pressure with a secondary effect on LV dP/dt_{max} was shown by the lack of responsiveness of LV dP/dt_{max} to acute increases in arterial pressure. In contrast to a defined transgenic model of increased contractility which is known to be due to increased β-adrenergic signaling (18), and in contrast to GH-deficient rats which have a similar phenotype (23), binding assays indicate that β receptor number is not increased in the IGF-$1^{m/m}$ mice. In the setting of increased adenylyl cyclase activity, the pattern of hemodynamic response is similar to that seen in mice transgenic for an inhibitor of the β adrenergic receptor kinase (βARK) (19); as in this model, the contractile performance of the IGF-$1^{m/m}$ mice can be further stimulated by isoproterenol, indicating that the additional recruitment of cardiac performance by β-adrenergic agonists is preserved in IGF-1 deficiency. The possibility of cross-talk between intracellular signaling pathways has been suggested for insulin and β adrenergic signaling in vitro (45); the present data support this hypothesis, and raise the possibilities that such interaction may be mediated through the IGF-1 receptor and involve the level or activity of the β adrenergic receptor kinase. This does not preclude additional increases in intrinsic cardiac myocyte contractility due to altered isoforms of contractile proteins such as α-actin and myosin heavy chain, of calcium regulatory proteins such as the troponins or phospholamban (which has also been implicated in the control of cardiac contractility in a transgenic model [46]), or by other increases in intracellular calcium or myofilament calcium sensitivity. The observation that β adrenergic coupling is enhanced without an increase in receptor density raises several interesting possibilities in light of the previous observations in GH-deficient rats. In the latter, presumptively also with secondary deficiency in IGF-1, a contribution of enhanced β adrenergic coupling to the increased contractility was not excluded. Thus GH and IGF-1 deficiencies synergistically enhance β adrenergic signaling by increases in β adrenergic receptor number and coupling, respectively; it is tempting to speculate that GH and IGF-1 excess might therefore inhibit β adrenergic signaling.

Cardiac hypertrophy. The lack of effect of IGF-1 deficiency on the adaptive hypertrophic response of the myocardium to the load imposed by transverse aortic constriction was unexpected. In light of evidence that IGF-1 produces a hypertrophic phenotype in cultured neonatal ventricular myocytes (25) as well as in vivo (9) and that IGF-1 mRNA and that for its receptor are upregulated in the ventricular myocardium following hemodynamic loading (6–8), it appeared likely that the hypertrophic response in vivo in the midi mice would at least be blunted. Instead, no effect was found, suggesting that either IGF-1 is not necessary for cardiac myocyte hypertrophy (or that other growth signals can compensate) or that the low levels of IGF-1 in the midi mice are sufficient (threshold effect).

Conclusions. Mice genetically deficient in IGF-1, yet with sufficient levels for normal survival, were generated by site-specific insertion of a disrupted exon 3. Alternative splicing of mRNA permitted low levels of intact IGF-1 synthesis. This severe IGF-1 deficiency resulted in animals with elevated systolic and diastolic blood pressure and augmented ventricular performance; nonetheless, cardiac chamber mass remained reduced in proportion to body mass. However, hypertrophic ventricular growth was not impaired in response to pressure overload. IGF-1–dependent pathways are thus implicated in the regulation of basal arterial blood pressure and cardiac contractility, yet normal IGF-1 levels are not required for adequate myocardial adaptation in response to sustained hemodynamic load. The mechanism by which IGF-1 deficiency affects basal blood pressure (such as modulation of vascular smooth muscle tone, alterations of endothelial function, or changes in neurohumoral tone producing an altered set point) remains to be elucidated; at least part of the increase in cardiac contractility, however, likely results from enhancement of basal adenylyl cyclase activity without an increase in β adrenergic receptor density, suggesting cross-talk between IGF-1 dependent and β adrenergic dependent signaling pathways. These IGF-$1^{m/m}$

mice should provide a model system in which to unravel these IGF-1 dependent pathways.

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