Disruption of growth hormone secretion alters Ca\(^{2+}\) current density and expression of Ca\(^{2+}\) channel and insulin-like growth factor genes in rat atria

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Larsen, Janice K., Chien-Chang Chen, and Philip M. Best. Disruption of growth hormone secretion alters Ca\(^{2+}\) current density and expression of Ca\(^{2+}\) channel and insulin-like growth factor genes in rat atria. Am J Physiol Heart Circ Physiol 288: H829–H838, 2005.—The influence of systemic GH/IGF-I secretion on cardiac LVA current density and expression of the cardiac low-voltage-activated (LVA) Ca\(^{2+}\) current in atrial myocytes isolated from adult hyperthyroid rats has been shown (47). These findings suggest that alterations in the GH/IGF-I axis can have significant effects on cardiac Ca\(^{2+}\) homeostasis. However, the role of the GH/IGF-I axis in regulating cardiac Ca\(^{2+}\) currents in normal cardiac development and in response to altered physiological states is less clear. Here, we have studied atrial myocytes isolated from adult rats stimulated to reenter an active growth phase by implantation of GH-secreting tumors (45). Thus there is a positive correlation between GH and IGF-I levels and the level of LVA Ca\(^{2+}\) current in cardiac cells, suggesting that the increased expression of cardiac LVA Ca\(^{2+}\) channels that accompanies enlargement of the heart is strongly influenced by the GH-IGF-I axis.

In an attempt to clarify the role of the GH-IGF-I axis in regulating cardiac Ca\(^{2+}\) current density, we have studied atrial Ca\(^{2+}\) currents and expression of Ca\(^{2+}\) channel \(\alpha_1\)-subunit genes in the spontaneous dwarf (SpDwf) rat. The SpDwf rat carries an autosomal recessive mutation in the GH gene that produces an abnormal splice variant resulting in premature translational termination (40). No GH is detected in the serum by radioimmunoassay or in the pituitary gland by immunocytochemistry (30, 40). Hepatic IGF-I mRNA is also dramatically reduced to <10% of that in normal rats (31). Therefore, the SpDwf strain provides a unique model to investigate the influence of systemic GH/IGF-I secretion on cardiac LVA Ca\(^{2+}\) channel expression. Our results show that the normal density of atrial LVA currents, as well as the abundance of mRNAs encoding Ca\(^{2+}\) channel \(\alpha_1\)-subunits, IGF-I, and IGF-I receptor (IGF-IR), is altered in this GH-deficient animal model and suggest that IGF-I produced in the heart may act in a paracrine or an autocrine manner to modulate expression of the LVA Ca\(^{2+}\) current in the atria.

The GH-deficient rat used in this study was initially identified with the acronym SDR (spontaneous dwarf rat, dr/dr) (30, 40). To differentiate the mutant animal more clearly from Sprague-Dawley rats, the strain from which the mutant arose and the strain that is used as control animals in this study, the acronym SpDwf is used in this report.

Expression of the cardiac low-voltage-activated (LVA) Ca\(^{2+}\) current varies during normal cardiac development and in response to altered physiological states. LVA Ca\(^{2+}\) currents are expressed in embryonic heart and postnatal atrial myocytes, but in many species they are small or absent in adult ventricular myocytes (15, 44) and are expressed differentially across the myocardial wall (42). Reexpression of LVA current and an increase in LVA Ca\(^{2+}\) current density are common findings in hypertrophied or enlarged cardiac muscle. Expression of LVA current is induced in ventricular myocytes from hypertrophied, pressure-overloaded hearts (25, 32) and after myocardial infarction (11), and both of these conditions are known to also stimulate expression of IGF-I (6, 12, 27). LVA current is also upregulated in atrial myocytes isolated from adult rats stimulated to reenter an active growth phase by implantation of GH-secreting tumors (45).

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MATERIALS AND METHODS

Materials. Oligonucleotides were constructed by Integrated DNA Technologies (Coraville, IA). Restriction endonucleases, DNase-free RNase, and Superscript prem amplification system were purchased from Life Technologies, GIBCO-BRL (Grand Island, NY); Taq DNA polymerase beads and RNase-free DNase I from Promega (Madison, WI); T7 polymerase and MEGAScript in vitro transcription kit from Ambion (Austin, TX); TA cloning kit, PCR2.1 vector system, and T4 DNA polymerase from Invitrogen (Carlsbad, CA); Ultraspec RNA isolation reagent from Biotex (Houston, TX); VistraGreen fluorescent DNA-binding dye from Amersham (Piscataway, NJ); methyl mercuric hydroxide from Crescent Chemical/Serva Fine Biochemicals (Hauppage, NY); DNA extraction spin columns from Millipore (Bedford, MA); collagenase B from Roche Molecular Biochemicals (Indianapolis, IN); rat tail collagen I and fibronectin from Sigma (St. Louis, MO); and IGF-I from Gropep (Adelaide, Australia).

Animals. SpDwf rats were obtained from an inbred colony maintained at the University of Illinois, Sprague-Dawley and SpDwf rats were housed with a 12:12-h light-dark cycle, and food and water were provided ad libitum. The care and use of the animals conform to all appropriate and required regulations of the University of Illinois and the US Government.

Myocyte isolation and culture. Atrial myocyte isolation was performed as previously described (33). Isolated atrial myocytes from Sprague-Dawley rats were plated at a density of 5 × 10⁴ per 35-mm petri dish in culture medium [50% DMEM, 50% Ham’s F-12, 4 mM insulin, 2% streptomycin-penicillin-ampicillin B (Fungizone), 2.5 mg/ml BSA, 1 mM selenium, 1 mM thymidine, 5 mM transferrin, and 10 mM testosterone] with 10% fetal bovine serum and kept in a humidified 5% CO₂ atmosphere at 37°C. Coverslips precoated with 10 μg/ml rat tail collagen I and 5 μg/ml fibronectin lined the bottom of the dishes. Cells were kept in the serum-containing medium for 48 h before serum was withdrawn. The concentration of insulin in the serum-free medium was chosen to promote binding to the insulin receptor (Kᵢ = 1 nM) with minimal binding to the IGF-IR (Kᵢ = 100 nM) (4). When IGF-I (recombinant human) and/or oligonucleotides were added to the cultures, the myocytes were first rinsed twice with Tyrode solution before addition of the peptide-containing, serum-free medium.

Antisense oligonucleotides. Cultured atrial myocytes were treated with 4 μM unmodified antisense oligonucleotides in the presence of 52 nM IGF-I for 24 h. Control cultures were treated with IGF-I only or IGF-I + mismatched oligonucleotides. The sequence for the antisense oligonucleotide targeting the IGF-IR transcript was 5’GAT AGT CGT TGC GGA TGT CA 3’, and the sequence for the mismatch oligonucleotide was 5’GAC AGA CTT CAG GAT TGT CA 3’ (36).

Electrophysiology. Ca²⁺ currents were recorded using the whole cell configuration of the patch-clamp technique, as previously reported (33). Patch pipettes, drawn from borosilicate glass, had resistances of 1–2.5 MΩ. Pipette capacitance was compensated electronically after seal formation. Cell capacitance and series resistance were calculated from the current transient induced by a hyperpolarizing pulse from −80 to −90 mV and compensated electronically.

LVA Ca²⁺ currents were isolated using trace subtraction from currents elicited at holding potentials of −90 and −50 mV. High-voltage-activated (HVA) currents were recorded from a holding potential of −50 mV. Current traces were corrected for linear capacitance and leak current using P/4 trace subtraction after each test pulse. Currents were sampled at 2.5–5 kHz with filtering at 1 kHz using an Axopatch 1D amplifier (Axon Instruments). The voltage dependence of activation and the rate of deactivation were determined by measuring the amplitude and rate of decay of tail currents produced by stepping from a depolarized conditioning potential back to −60 mV. Conditioning pulses up to −40 mV activated LVA currents exclusively, and tail currents were best fit by a single exponential with a time constant of ~5 ms. For conditioning pulses above −40 mV, HVA and LVA currents were activated, and deactivation was best fit using a double exponential with time constants of ~5 and <1 ms (Table 1). For activation curves, currents elicited at various conditioning potentials were expressed as a fraction of the maximal current amplitude, and the resulting data were fit using the Boltzmann equation.

Table 1. Ca²⁺ current properties in control and SpDwf atrial myocytes at 4.5 wk

<table>
<thead>
<tr>
<th>Current</th>
<th>Control</th>
<th>SpDwf</th>
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</thead>
<tbody>
<tr>
<td>LVA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to peak at −30 mV, ms</td>
<td>9.2 ± 0.8 (5)</td>
<td>8.3 ± 0.6 (7)</td>
</tr>
<tr>
<td>Vₛ, mV</td>
<td>Activation</td>
<td>−35.3 (3)</td>
</tr>
<tr>
<td>HVA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to peak at +10 mV, ms</td>
<td>4.8 ± 0.8 (6)</td>
<td>6.3 ± 0.4 (10)</td>
</tr>
<tr>
<td>Vₛ, mV</td>
<td>Activation</td>
<td>−3.7 (3)</td>
</tr>
</tbody>
</table>

Tail currents were analyzed to measure voltage of half-maximal activation (Vₛ); time to peak and time constant of deactivation (τ) were measured from whole cell Ca²⁺ currents of acutely isolated atrial myocytes. LVA and HVA, low- and high-voltage-activated. Number of individual experiments is indicated in parentheses. Ca²⁺ current properties in SpDwf rats were not significantly different from aged-matched controls.
Results

Whole body and heart weights in SpDwf rats are severely reduced. Compared with controls, SpDwf rats show a significant retardation in the rate and extent of postnatal growth. At 10 wk, SpDwf rats weighed ~20% of age-matched controls (Fig. 1A). The dramatic peak in growth rate at 4.5 wk in normal animals was severely blunted in SpDwf rats, with a small peak in growth rate at 3 wk (Fig. 1B). However, the tight correlation between whole body weight and heart weight is maintained in the SpDwf animals (Fig. 1C). Heart weight-to-body weight ratio of SpDwf rats was ~0.005, a value similar to control rats (9, 44).

Ca\textsuperscript{2+} currents recorded from atrial myocytes isolated from SpDwf rats have normal biophysical and pharmacological properties. HVA and LVA Ca\textsuperscript{2+} currents are present in atrial myocytes isolated from SpDwf rats. Typical Ca\textsuperscript{2+} current traces are shown in Fig. 2 for an atrial myocyte isolated from a 4.5-wk-old SpDwf animal. At a holding potential of −90 mV, the currents recorded at more negative test potentials (−50 to −30 mV) showed rapid activation and inactivation kinetics (Fig. 2A), typical of cardiac LVA currents. At a test potential of −10 mV, a second, larger component of current was seen that inactivates more slowly. The LVA current was inactivated at depolarized holding potentials, allowing separation of the currents by subtraction of current records elicited at the same test potential but from different holding potentials. This protocol was used to measure peak currents under nonsynchronized conditions to generate current-voltage relations for the LVA and HVA currents in these cells (Fig. 2B). Because current activation and inactivation are time- and voltage-dependent processes, a more accurate measure of the biophysical characteristics of the LVA and HVA currents is obtained by an analysis of tail currents. Therefore, we used tail current analysis to measure the voltage dependence of activation and the rate of deactivation of atrial Ca\textsuperscript{2+} currents in myocytes isolated from SpDwf and control rats (Fig. 2, C–E). Analysis of the tail currents recorded from atrial myocytes from SpDwf rats showed no difference from those recorded from control animals (Fig. 2, C and D, Table 1). The average voltage of half-maximal activation, time to peak, and time constant of deactivation for the atrial HVA and LVA Ca\textsuperscript{2+} currents were not significantly different in SpDwf rats and age-matched controls (Table 1).

The Ca\textsuperscript{2+} currents in SpDwf rats showed typical responses to pharmacological agents known to affect cardiac HVA and LVA currents. Both currents were blocked by Cd\textsuperscript{2+} (2 mM), a nonspecific Ca\textsuperscript{2+} channel blocker. An L-type channel agonist, BAY K 8644 (1 mM), increased the HVA current by fivefold, whereas LVA current was unaffected. LVA current was significantly blocked by Ni\textsuperscript{2+} (38 ± 13% block at 200 μM, n = 3) and mibebradil [35 ± 10% at 8 μM (n = 3) and 70 ± 22% at 25 μM (n = 3)].

LVA Ca\textsuperscript{2+} current density is elevated in atrial myocytes isolated from SpDwf animals. In the normal rat, atrial LVA Ca\textsuperscript{2+} current density decreases postnatally, with densities in adults approximately one-third of those in young animals (22, 44). This decrease is coincident with the decrease in serum GH concentration that occurs during aging. To test the hypothesis that atrial LVA current expression is coupled to the levels of GH or IGF-I in the blood, we determined whether the normal expression and age-related decline of LVA Ca\textsuperscript{2+} current density are disrupted by the elimination of GH production and by the drastic reduction of hepatic IGF-I in the SpDwf mutants. If
circulating GH or IGF-I directly controls LVA current expression, the current should be greatly diminished in atria of SpDwf rats.

Ca\(^{2+}\) current densities were measured in postnatal atrial myocytes isolated from 3- to 12-wk-old SpDwf rats as well as aged-matched control rats. In postnatal SpDwf atrial myocytes, LVA current densities remained constant at \(\sim 1\) pA/pF (Fig. 3A), a level similar to the highest values observed in myocytes isolated from controls (44). In atrial myocytes from control animals, the density of LVA Ca\(^{2+}\) current at 5 wk of age was significantly higher than that in myocytes isolated from 8-wk-old animals (Fig. 3A; 1.2 and 0.55 pA/pF at 5 and 8 wk, respectively, \(P < 0.05\)), consistent with the previously documented decay of LVA current during normal postnatal development (44). Atrial HVA current density did not differ between control and SpDwf rats and did not vary significantly as a function of postnatal age (Fig. 3B), also consistent with previous reports from control animals (44).

Thus the disruption of GH production and drastic reduction in hepatic IGF-I secretion in the SpDwf rat did not lead to a decrease in atrial LVA current expression, as might have been expected from the known effects of these hormones on current expression in normal animals (33).

Disruption of the GH-IGF-I axis alters postnatal expression of mRNAs encoding some, but not all, Ca\(^{2+}\) channel \(\alpha_1\)-subunits. Four genes encoding Ca\(^{2+}\) channel \(\alpha_1\)-subunits (Ca.1,2, Ca.2,3, Ca.3,1, and Ca.3,2) are known to be expressed in all chambers of the rat heart (19). A fifth gene (Ca.1,3) appears to be exclusively expressed in the sinoatrial (SA) node and surrounding atrial myocytes but is not seen in the ventricle (24, 26, 41, 46, 47). Although we clearly detected transcripts for Ca.1,2, Ca.2,3, Ca.3,1, and Ca.3,2 in postnatal SpDwf atrial tissue, we were unable to detect Ca.1,3 mRNA. To further characterize and quantitate the effect of the GH-IGF-I axis on the abundance of the mRNAs encoding Ca.1,2, Ca.2,3, Ca.3,1, and Ca.3,2 pore-forming subunits, we employed quantitative RT-PCR.

The most abundant Ca\(^{2+}\) channel \(\alpha_1\)-subunit mRNA in SpDwf and normal rat atria was Ca\(_{\text{v}1.2}\) (2.86–3.48 \(\times 10^6\) mol mRNA/\(\mu\text{g total tissue RNA}\)), which is known to encode the

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**Fig. 2.** High- and low-voltage-activated (HVA and LVA) Ca\(^{2+}\) currents expressed in atrial myocytes of SpDwf rats. A: whole cell Ca\(^{2+}\) current measured under nonisochronic conditions from atrial myocyte from a 4.5-wk-old SpDwf rat. Ca\(^{2+}\) currents were elicited from holding potentials of \(-90\) mV using test pulses from \(-50\) to \(-10\) mV in 10-mV steps. B: current density (I) plotted against depolarization voltages (V) for HVA and LVA Ca\(^{2+}\) currents from SpDwf rats. LVA Ca\(^{2+}\) current was activated at less positive potentials than HVA current. Hp, holding potential. C: representative tail current recorded from atrial myocyte from 4.5-wk-old SpDwf rat. D and E: activation curves for atrial LVA and HVA Ca\(^{2+}\) currents isolated from control (Con) and SpDwf rats, expressed in terms of relative conductance (g/g\(_{\text{max}}\)). No significant difference was measured in voltage of half-maximal activation between SpDwf and control cells.
pore-forming subunit of the HVA or L-type Ca\(^{2+}\) channel in the heart. Although there was a small, but significant, increase in Cav1.2 mRNA content in atria from 4- and 5-wk-old SpDwf rats (P < 0.05, by Student’s t-test) compared with age-matched controls, Cav1.2 mRNA was expressed at relatively constant amounts in atria at all postnatal ages studied (Fig. 4A).

Rat cardiac cells also express two genes, Cav3.1 and Cav3.2, which encode the pore-forming subunits of LVA currents. In SpDwf rats, Cav3.1 message was expressed at all ages, with a level ~10-fold lower than that for Cav1.2 (0.86–1.09 \times 10^{5} mol mRNA/\mu g total tissue RNA). The abundance of atrial Cav3.1 mRNA in controls was statistically equivalent to that found in SpDwf rats, except at 3 wk of age (Fig. 4C; P < 0.05, by Student’s t-test). In SpDwf and control atria, the abundance of Cav3.1 message declined only slightly with advancing age, reaching significance at 10 wk (P < 0.05, by Dunnett’s ANOVA). The expression pattern of Cav3.2 mRNA varied dramatically as a function of postnatal age. In SpDwf rats, Cav3.2 mRNA was present only at 3 wk of age (1.09 \times 10^{5} mol mRNA/\mu g total tissue RNA) and was undetectable thereafter (Fig. 4D). There was also an abrupt disappearance of Cav3.2 mRNA in control animals, although this occurred 1 wk later.

The Ca\(_{2.3}\) gene is thought to encode a drug- and toxin-resistant Ca\(^{2+}\) (R-type) current. The abundance of Cav2.3 mRNA in SpDwf atria ranged from 1.35 to 1.77 \times 10^{5} mol mRNA/\mu g total tissue RNA (Fig. 4B). In the SpDwf rat atria, there was a slight decline in expression of this transcript, although no significant difference was found in the abundance of Cav2.3 message at any postnatal age. In controls, however, the abundance of Cav2.3 message declined with postnatal age (P < 0.05, by Dunnett’s ANOVA), as previously reported (19). Thus, at 10 wk, the abundance of Cav2.3 message had fallen 35% in controls but had not significantly declined in SpDwf rats (Fig. 4B). At 6 and 10 wk, the absolute level of Cav2.3 mRNA was 1.8- to 2.3-fold higher in SpDwf rats than in age-matched controls, respectively (P < 0.05, by Student’s t-test).

Thus disruption of GH secretion in the SpDwf rat alters the abundance of mRNAs encoding only two (Cav2.3 and Cav3.2) of the four Ca\(^{2+}\) channel \(\alpha\)-subunit genes expressed in the rat atria. The normal postnatal decline in abundance of Cav2.3 transcript in atria is eliminated in SpDwf animals, whereas the normal decline in Cav3.2 expression is accelerated.

Disruption of GH secretion alters local expression of mRNAs encoding IGF-I and IGF-IR. One possible mechanism to explain the unexpected increase in LVA current density in atrial myocytes isolated from SpDwf rats is compensation for the decrease in circulating GH/IGF-I by changes in local IGF-I production in the heart. It is clear that disruption of GH production results in a reduction of hepatic IGF-I mRNA in SpDwf rats (31); however, it is unknown whether this disruption also influences local IGF-I transcript levels in the heart. Therefore, quantitative RT-PCR was used to monitor changes in the abundance of mRNA transcripts encoding IGF-I and IGF-IR in postnatal atrial tissue.

The expression pattern of IGF-IR mRNA isolated from SpDwf rats was altered in two ways compared with the expression pattern in controls (Fig. 4E): 1) the absolute abundance (averaging 6.1 \pm 0.4 \times 10^{5} mol mRNA/\mu g total tissue mRNA in SpDwf atria) was significantly elevated compared with controls at 4, 6, and 10 wk of age, and 2) the decrease in IGF-IR mRNA measured in controls as a function of postnatal age (P < 0.05, by Dunnett’s ANOVA) was absent in the SpDwf animals. For the IGF-I transcript, the abundance of this message in control animals declined with advancing age, with no measurable IGF-I mRNA found in atrial tissue from 10-wk-old rats (Fig. 4F). However, no significant postnatal changes in IGF-I mRNA were measured in atria from SpDwf rats, resulting in robust expression at 10 wk. In aged-matched animals, IGF-I mRNA was elevated in 4- and 10-wk-old SpDwf animals compared with controls, whereas tissue from 3-wk-old animals contained significantly lower levels of IGF-I mRNA than tissue from controls (P < 0.05, by Student’s t-test).

Thus the decrease in GH production in the SpDwf animals is accompanied by significant increases in the local abundance of mRNA transcripts encoding IGF-I and IGF-IR in the atria. Specifically, IGF-I mRNA is elevated significantly in older animals, and the expression of mRNA encoding IGF-IR is also increased at most postnatal ages.

**IGF-I enhances LVA, but not HVA, Ca\(^{2+}\) current density in cultured atrial myocytes.** Because the disruption of GH production in the SpDwf animals was accompanied by significant changes in the abundance of mRNA transcripts encoding IGF-I and IGF-IR, it seems likely that local IGF-I production is
Fig. 4. Quantitation of Ca\(^{2+}\) channel \(\alpha_1\)-subunit, as well as insulin-like growth factor I (IGF-I) and IGF-I receptor (IGF-IR), mRNAs in SpDwf animals as a function of postnatal development. Total RNA was extracted from atria of SpDwf and control rats, and amounts of transcripts for Cav1.2 (A), Cav2.3 (B), Cav3.1 (C), Cav3.2 (D), IGF-IR (E), and IGF-I (F) were calculated by quantitative RT-PCR. A: amount of atrial Cav1.2 mRNA increased slightly above control rats at 4 and 5 wk of age, but amount of transcript did not vary statistically during postnatal development from 3 to 10 wk of age in control or SpDwf animals.

B: abundance of atrial Cav2.3 remained significantly elevated in 6- and 10-wk-old SpDwf animals compared with 4-wk-old animals. In older control rats, amount of Cav2.3 transcript declined to ~35% compared with 4-wk-old animals. C: levels of transcript encoding Cav3.1 in SpDwf atria were very similar to control animals, except at 3 wk, when SpDwf animals showed a slight, but significant, decrease in levels of this mRNA. D: mRNA for Cav3.2 was significantly more abundant in control than in SpDwf atrial myocytes at 3 and 4 wk of age. Levels of Cav3.2 declined dramatically to undetectable levels as early as 4 wk of age. E: mRNA encoding IGF-IR was significantly upregulated in SpDwf atria at 4, 6, and 10 wk of age compared with controls. F: IGF-I mRNA was also expressed at higher levels of abundance in atria from 4- and 10-wk-old SpDwf rats than in atria from normal animals. mRNA expression of IGF-I was significantly lower in SpDwf atria at 3 wk of age. No amplification of IGF-I transcript was observed in 10-wk-old control atria. Values are means ± SE; \(n = 8–12\). *Significantly different from control at age-matched time points, \(P < 0.05\) (by Student’s \(t\)-test). **Significantly different from 4-wk-old animals as a function of postnatal development, \(P < 0.05\) (by Dunnett’s ANOVA). Control data of Ca\(^{2+}\) channel \(\alpha_1\)-subunit transcripts are replotted from a previous study (19).

Acutely isolated atrial myocytes were maintained in serum-free culture conditions for 2 days. After 2 days, human recombinant IGF-I (52 nM) was added to the culture medium. The amplitude of LVA Ca\(^{2+}\) current recorded from cells exposed to IGF-I was significantly increased compared with currents recorded from untreated cells (Fig. 5). The IGF-I-dependent increase in LVA current was clearly observed in the current density-voltage relation and when LVA and HVA Ca\(^{2+}\) currents were separated by subtraction of current records elicited from different holding potentials. Peak LVA Ca\(^{2+}\) current recorded at \(-30\) mV was \(-25.0\) and \(-39.8\) pA for control and treated cells, respectively (Fig. 5B). On average, LVA Ca\(^{2+}\) current density in 2 mM Ca\(^{2+}\) increased from \(-0.48 \pm 0.04\) (\(n = 5\) cells) to \(-0.79 \pm 0.02\) (\(n = 17\) cells) after 24 h of IGF-I treatment (\(P < 0.05\)). Average current density increased further from \(-0.56 \pm 0.14\) (\(n = 4\) cells) to \(-1.2 \pm 0.23\) (\(n = 7\) cells) after 48 h of IGF-I treatment (\(P < 0.01\)). The 24-h IGF-I treatment, however, did not affect LVA current in time to peak (9.2 \pm 1.9 ms, \(n = 10\) cells) or the time constant of inactivation (22.9 \pm 3.3 ms, \(n = 10\) cells, test potential \(= -30\) mV). Additionally, the voltage dependence of activation did not vary after the addition of IGF-I for 24 or 48 h (Table 2). The slope factor was unaltered at 24 h but showed a significant difference after 48 h (Table 2).

Neither peak HVA Ca\(^{2+}\) current density (Fig. 5) nor cell capacitance (data not shown) was significantly different in IGF-I-treated and control cells at any time. Treatment of cells with IGF-I that was inactivated by boiling had no effect on peak LVA current (\(-0.42 \pm 0.03\), \(n = 7\) cells), HVA current (\(-5.14 \pm 0.39\), \(n = 13\) cells), or cell capacitance compared with controls. These results demonstrate that the IGF-I effects were specific for the LVA Ca\(^{2+}\) current.
IGF-I-dependent increase in LVA Ca\(^{2+}\) current density occurs within 8–24 h at physiological concentrations of hormone. A concentration-response curve was compiled to determine the concentration at which IGF-I exerts its effects. The enhancement of LVA Ca\(^{2+}\) current density was concentration dependent (Fig. 6A). Maximal enhancement occurred at 52 nM, and the approximate concentration for half-maximum stimulation was 5.0 nM.

A time course was conducted to determine whether the increases in LVA current density occurred immediately or developed over longer periods of time (Fig. 6B). LVA Ca\(^{2+}\) current densities were measured 0.5, 3, 8, and 24 h after the addition of 52 nM IGF-I. A significant increase in LVA current was measured after 8 h and lasted for up to 24 h. Although a slight increase in LVA Ca\(^{2+}\) current density was observed in cells treated with IGF-I for 3 h, it was not statistically different from the starting value. Cell capacitance did not change during this time.

**IGF-I enhances LVA current via IGF-IR.** To test whether the effects of IGF-I are mediated through the IGF-IR, an antisense

Table 2. Parameters of steady-state activation of Ca\(^{2+}\) channels in cultured atrial myocytes

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 5 + IGF-I</th>
<th>Day 6</th>
<th>Day 6 + IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>V(_{0.5}), mV</td>
<td>-2.57±1.14 (12)</td>
<td>-4.7±1.3 (10)</td>
<td>-6.1±1.2 (18)</td>
<td>-8.14±1.2 (6)</td>
<td>-5.87±1.5 (9)</td>
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<td>K, mV</td>
<td>-7.4±0.24 (12)</td>
<td>-7.4±0.3 (9)</td>
<td>-6.8±0.2 (18)</td>
<td>-7.2±0.44 (6)</td>
<td>-7.8±0.6 (9)</td>
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<td>LVA</td>
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<tr>
<td>V(_{0.5}), mV</td>
<td>-36.6±2.8 (3)</td>
<td>-38.6±3.0 (3)</td>
<td>-35.6±1.5 (10)</td>
<td>-34.3±3.2 (3)</td>
<td>-36.6±2.9 (3)</td>
</tr>
<tr>
<td>K, mV</td>
<td>-7.2±2.0 (3)</td>
<td>-4.6±0.4 (3)</td>
<td>-6.4±0.6 (10)</td>
<td>-2.3±0.1 (3)</td>
<td>-5±1.4 (3)*</td>
</tr>
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</table>

Values are means ± SE of number of individual experiments in the parentheses. Activation curves were plotted from amplitude of tail currents measured at -60 mV. Data were fit using Boltzmann equation. Atrial myocytes were cultured for 2 days with serum-containing medium. On day 3, cultured medium was switched to serum-free conditions and maintained up to day 6. Peak current at various test potentials for HVA and LVA Ca\(^{2+}\) channels were measured from atrial myocytes maintained in serum-free conditions on day 4 up to day 6 with and without addition of insulin-like growth factor I (IGF-I) for 24 h (day 5 + IGF-I) or 48 h (day 6 + IGF-I). For HVA Ca\(^{2+}\) channels, neither V\(_{0.5}\) nor slope factor (K) was significantly altered at any time in culture or after addition of IGF-I. For LVA Ca\(^{2+}\) channel, myocytes held in culture for 6 days after a 48-h IGF-I treatment showed an altered slope factor (*P < 0.05); however, no difference in V\(_{0.5}\) was measured.
oligonucleotide strategy was employed. Treatment for 24 h with IGF-I + antisense oligonucleotide targeting IGF-IR transcript significantly decreased LVA Ca^{2+} current density by 40% compared with IGF-I treatment (P < 0.05; Fig. 7). As expected, the same treatments had no effect on HVA Ca^{2+} current density or cell capacitance. When we controlled for nonspecific effects, mismatched oligonucleotide had no effect on either Ca^{2+} current (Fig. 7).

**DISCUSSION**

We have used the SpDwf rat to evaluate the influence of the GH-IGF-I axis on expression of atrial Ca^{2+} currents and Ca^{2+} channel α_{1y}-subunit genes during postnatal development. Robust LVA currents are routinely recorded from atrial myocytes but are generally absent in ventricular myocytes isolated from postnatal animals. Expression of the atrial LVA Ca^{2+} current declines during postnatal development, a time course that parallels the normal decrease in plasma GH as the animal matures (44). When plasma GH levels are experimentally elevated in adult rats, the animals reenter an active growth phase, with an associated increase in cardiac muscle mass. An increase in cardiac growth is preceded by a dramatic increase in expression of LVA Ca^{2+} current, which is again limited to the atria (45). No increase in LVA current is seen in ventricular myocytes under these conditions (45). It has been assumed that the increase in LVA Ca^{2+} current is caused by an indirect effect of GH, stimulating the release of IGF-I from the liver, thus elevating circulating IGF-I concentrations (45). However, the possible contribution of cardiac-specific IGF-I production or a direct effect of GH on cardiac tissue has not been investigated. The present study was designed to address these possibilities.

Elimination of circulating GH and IGF-I of hepatic origin in the SpDwf rat alters the normal postnatal expression pattern of the LVA current. One possible explanation for our results is that changes in local IGF production in the heart compensate for the lack of circulating plasma GH and IGF levels, thus maintaining expression of the atrial LVA Ca^{2+} current. It is thus proposed that compensatory upregulation of IGF-I and its receptor in the atria of the SpDwf animals accounts for the alteration in atrial LVA current expression in these GH-null animals. Support for this scenario comes from our observation that the abundance of mRNA encoding IGF-I and IGF-IR in the atria is increased in the SpDwf rats at most postnatal ages. Although the presence of mRNA transcripts does not guarantee expression of functional protein, it has been shown that most newly transcribed IGF mRNAs are translated into IGF proteins (7). If it is assumed that the observed increases in mRNA levels are accompanied by increases in IGF-I and IGF-IR protein, it is reasonable to propose that the increased availability of IGF-I and IGF-IR in the atria regulate the expression of LVA current and compensate for the lack of circulating GH and IGF-I.

Using cultured cells, we have shown that physiological concentrations of IGF-I cause a two- to threefold increase in LVA Ca^{2+} current density. The increase in atrial LVA Ca^{2+} current density stimulated by IGF-I developed over several hours, suggesting that the observed increase in current density is due to newly synthesized protein. This notion is consistent with previously reported effects of IGF-I and other growth factors (2, 21, 38). The magnitude of the increase in LVA Ca^{2+} current density is similar to that in cells isolated from animals with increased serum GH levels (45). This stimulatory effect is specific for the LVA Ca^{2+} current, inasmuch as, in the whole animal studies (45), HVA Ca^{2+} currents were not affected. From our data, we estimate the concentration of IGF-I that results in a half-maximal increase of LVA Ca^{2+} current in atrial myocytes to be ~5.0 nM. This is similar to concentrations reported for the induction of other physiological effects (39) and to the reported binding affinity of IGF-I to the IGF-IR (4). We used an antisense oligonucleotide strategy to demonstrate that this effect is mediated through IGF-IR. Antisense oligonucleotides have been successfully used to specifically block translation of the targeted transcripts in cardiomyocytes (16, 33). An antisense oligonucleotide targeting the IGF-IR specifically blocked the induction of LVA Ca^{2+} current density by IGF-I but had no effect on HVA Ca^{2+} current density. Therefore, the IGF-I-dependent increase in LVA Ca^{2+} current in atrial myocytes appears to be due to specific binding of IGF-I to IGF-IR.

Because atrial LVA current remains elevated during postnatal development in myocytes isolated from SpDwf rats, in contrast to the decrease in controls, it was interesting to compare the abundance of mRNAs encoding Ca^{2+} channel α_{1y}-subunits in these two rat strains. The Ca_{3.1} and Ca_{3.2} genes are thought to encode LVA Ca^{2+} channels, and both are expressed in cardiac cells. Normally, the abundance of the Ca_{3.2} transcript decreases rapidly soon after birth (29) and is beyond detectable limits by 5 wk (19). In the absence of GH production, this decline begins earlier (this study). Because expression of the Ca_{3.2} gene is undetectable in rat atria after 3 or 4 wk of age, it is unlikely that this pore-forming subunit contributes to the LVA current recorded from myocytes isolated from older animals. In contrast, the abundance of the Ca_{3.1} mRNA transcript did not differ statistically between the SpDwf (this study) and normal atrial myocytes (19). The contribution of the Ca_{3.1}, rather than the Ca_{3.2}, transcript to the atrial LVA Ca^{2+} current in SpDwf rats is supported by the finding that the LVA current (at 4.5 wk) was sensitive to Ni^{2+} in the 200 μM range, as has been reported for currents arising from the expression of Ca_{3.1} (20). A more thorough pharmacological and kinetic analysis is required before it can be concluded that only a single α_{1y}-subunit contributes to the LVA current. Furthermore, if the transcript encoding the Ca_{3.1}
protein is responsible for most of the atrial LVA current in normal and SpDwf rats, a posttranscriptional modification would be required to explain the decline of LVA current in control animals that is not seen in the SpDwf rats.

The significance of the increase in mRNA encoding Ca\textsubscript{v}2.3 in the atria of the SpDwf rat is not understood. It has been shown that a decrease in Cav2.3 mRNA begins at or shortly after birth in normal rats (22) and declines to \textasciitilde 35% of maximum in 6- and 10-wk-old animals (19). This decrease parallels the decrease in atrial LVA current that occurs during postnatal growth (44). In the SpDwf rats, this decline is severely attenuated as Ca\textsubscript{v}2.3 mRNA abundance is maintained postnatal growth (44). In the SpDwf rats, this decline is parallels the decrease in atrial LVA current that occurs during maximum in 6- and 10-wk-old animals (19). This decrease.

Ca\textsuperscript{2+} channels is required for platelet-derived growth factor-induced fibroblast replication by promoting progression to the S phase (43). An association between the elevated LVA Ca\textsuperscript{2+} current (and lowered HVA Ca\textsuperscript{2+} current) and the S phase of the cell cycle was also reported in cultured smooth muscle and neonatal ventricular myocytes (10, 17). Common to all these reports is a strong correlation between expression of LVA Ca\textsuperscript{2+} channels and cell growth.

It is interesting to note that, during cardiac growth resulting from elevated plasma GH, LVA Ca\textsuperscript{2+} current is elevated in atrial, but not ventricular, myocytes (44, 45). In contrast, LVA Ca\textsuperscript{2+} current is reexpressed in ventricular myocytes of adult animals during the hypertrophic response resulting from altered hemodynamics (25, 32). This suggests that the regulatory mechanisms controlling LVA Ca\textsuperscript{2+} current expression in the atria and ventricles are different or activated differentially via IGF-I (atria) or altered hemodynamics (ventricle).

In conclusion, disruption of the systemic secretion of GH and hepatic IGF-I results in compensatory changes in the local production of atrial IGF-I and IGF-IR, as well as in changes in expression of atrial LVA Ca\textsuperscript{2+} current and atrial Ca\textsuperscript{2+} channel \(\alpha\textsubscript{1}L\)-subunits. The results suggest that IGF-I produced in the atria acts in a paracrine/autocrine manner to compensate for lower levels of circulating IGF-I and, thus, regulates expression of LVA Ca\textsuperscript{2+} current independently of GH. These results indicate the importance of IGF-I in regulating cardiac function by altering Ca\textsuperscript{2+} current expression.

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