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Cardiac Function in Mice Lacking the Glucagon-Like Peptide-1 Receptor

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Glucagon-like peptide-1 (GLP-1) acts via its G protein-coupled receptor (GLP-1R) to regulate blood glucose. Although the GLP-1R is widely expressed in peripheral tissues, including the heart, and exogenous GLP-1 administration increases heart rate and blood pressure in rodents, the physiological importance of GLP-1R action in the cardiovascular system remains unclear. We now show that 2-month-old mice with genetic deletion of the GLP-1R (GLP-1R$^{-/-}$) exhibit reduced resting heart rate and elevated left ventricular (LV) end diastolic pressure compared with CD-1 wild-type controls. At the age of 5 months, echocardiography and histology demonstrate increased LV thickness in GLP-1R$^{-/-}$ mice. Although baseline hemodynamic parameters of GLP-1R$^{-/-}$ did not differ significantly from those of wild type, GLP-1R$^{-/-}$ mice displayed impaired LV contractility and diastolic function after insulin administration. The defective cardiovascular response to insulin was not attributable to a generalized defect in the stress response, because GLP-1R$^{-/-}$ mice responded appropriately to insulin with increased c-*fos* expression in the hypothalamus and increased circulating levels of glucagon and epinephrine. Furthermore, LV contractility after exogenous epinephrine infusion was also reduced in GLP-1R$^{-/-}$ mice. These findings provide new evidence implicating an essential role for GLP-1R in the control of murine cardiac structure and function *in vivo*. (*Endocrinology* 144: 2242–2252, 2003)

THE PROGLUCAGON GENE is expressed in the pancreatic islets, gut endocrine cells, and brain, and gives rise to structurally related peptide hormones, the proglucagon-derived peptides, that exert a wide range of actions converging on regulation of energy homeostasis (1). Pancreatic glucagon, liberated from islet A cells by the action of prohormone convertase-2, regulates plasma glucose via effects on hepatic glycogenolysis and gluconeogenesis. In the gut, prohormone convertase-1 mediates the liberation of glicentin, oxyntomodulin, intervening peptides, and two glucagon-like peptides, GLP-1 and GLP-2. Although the biological importance of glicentin and oxyntomodulin remains uncertain, GLP-2 acts in the gut to maintain the integrity and function of the mucosal epithelium (2, 3), and GLP-1 regulates nutrient flux via effects on gastrointestinal motility, glucagon and insulin secretion, and the control of appetite and body weight (BW; Refs. 4 and 5).

The actions of the proglucagon-derived peptides are mediated by distinct members of the glucagon-secretin receptor superfamily that transduce their effects via dual signaling pathways, including activation of adenylate cyclase and intracellular calcium flux (6, 7). The GLP-1 receptor (GLP-1R) is widely expressed in islet cells, kidney, lung, brain, heart, and the gastrointestinal tract (8–10). In contrast, the GLP-2 receptor is more restricted in its tissue distribution (11), because GLP-2R mRNA transcripts are predominantly localized to the brain and gastrointestinal tract (12, 13).

The observations that GLP-1 exerts multiple complementary actions that lower blood glucose in subjects with type 2 diabetes has engendered considerable interest in the possibility that long-acting GLP-1 analogs may be useful for the treatment of diabetes mellitus (1, 14, 15). However, GLP-1 also exerts actions distinct from those focused on glucoregulation, including regulation of hypothalamic and pituitary neuroendocrine function (16), reduction of pulmonary vascular tone (17), and stimulation of tracheal mucous secretion (18). Indeed, peripheral or central GLP-1 agonist administration is associated with a rapid increase in heart rate (HR) and blood pressure (BP) in rodents and calves (19–21).

GLP-1 has been shown to activate cAMP in isolated rat cardiac myocytes (22), but it also activates central and peripheral autonomic responses (21). Because GLP-1 receptor RNA transcripts are detected in the rodent and human heart (10, 23) and in regions of the brain involved in autonomic function (21, 24, 25), central or peripheral GLP-1R signaling systems may transduce direct and indirect cardiovascular effects of circulating GLP-1. However, despite evidence implicating exogenous GLP-1 administration in the regulation of cardiac contractile and chronotropic activity (20, 21, 26, 27), whether central or peripheral GLP-1R systems are essential for cardiovascular function has not been established. To assess the importance of endogenous cardiac GLP-1R

Abbreviations: BP, Blood pressure; BW, body weight; DBP, diastolic BP; dP/dT, first derivative of left ventricle pressure; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; H&E, hematoxylin and eosin; HR, heart rate; HW, heart weight; icv, intracerebroventricular; LV, left ventricular; LVEDP, LV end diastolic pressure; LVEDV, LV end-diastolic volume; LVSP, LV systolic pressure; PLT, posterolateral thickness; PVN, paraventricular nucleus; SBP, systolic BP; ST, septal thickness; WT, wild-type.

activity for baseline cardiovascular function *in vivo*, we first conducted invasive hemodynamic monitoring in male 2-month-old mice with genetic disruption of GLP-1R (GLP-$1R^{-/-}$; Ref. 28) and age- and gender-matched CD-1 wild-type (WT) controls. We next used echocardiography, cardiac histology, and invasive hemodynamic monitoring to assess cardiac structure and function in GLP-$1R^{-/-}$ and WT animals at 5 months of age. Finally, cardiac hemodynamic responses to external stress hormones were compared in older GLP-$1R^{-/-}$ mice and age-matched WT controls. Our data suggest a heretofore-unrecognized role for GLP-1R signaling in cardiac function, which is most evident in the cardiovascular response to stress.

Materials and Methods

Animals

All animal experimentation was carried out in accordance with protocols and guidelines approved by the Animal Care Committee at the Toronto General Hospital. Two- and 5-month old male GLP-$1R^{-/-}$ mice in the CD-1 genetic background and age- and sex-matched control CD-1 WT mice were maintained in the Toronto General Hospital Animal Facility on normal rodent chow and genotyped by Southern blot analysis as previously described (28). Animals younger than 2 months of age, particularly those lacking the GLP-1R, were found to be too small for safe and reproducible carotid artery cannulation. Moreover, they demonstrate less stable thermoregulation and hemodynamic values after anesthesia. Hence, invasive hemodynamic studies were carried out in older mice.

Cardiac hemodynamic measurements and drug administration

Mice were anesthetized using ketamine-HCl (100 mg/kg ip) and xylazine-HCl (10 mg/kg ip) and placed on a warming pad. A mild cardiodepressant effect of this anesthesia has been described (29). The right common carotid artery and/or jugular vein was isolated after midline neck incision and cannulated using a Millar Mikro-tip pressure transducer (1.4F sensor, 2F catheter; Millar Instruments, Houston, TX) as previously described (30). From jugular vein cannulations, the catheter was advanced to the right atrium, passed through the tricuspid valve, and stabilized in the right ventricular cavity. From carotid artery cannulations, stable arterial pressure tracings were recorded, from which systolic and diastolic blood pressures (SBP and DBP) were later measured. The catheter was advanced to the aorta, passed through the aortic valve, and stabilized in the left ventricular (LV) cavity. HR (beats per minute), systolic and diastolic LV pressures (mm Hg) were recorded at baseline and specific time intervals up to 10 min after the administration of drugs (see *Results*). Peak positive and negative first derivatives (maximum ± dP/dT; mm Hg/second) were obtained from LV pressure curves using Origin 6.0 (Microcal Software, Inc., Northampton, MA). Once a stable baseline for cardiovascular parameters was observed, administration of drugs was initiated. Weight-adjusted doses of insulin (3 U/kg, ip; Humulin R, Eli-Lilly Canada, Toronto, Canada) and adrenaline (1 μg/kg iv, Warner-Lambert, Toronto, Canada) were administered. Blood glucose levels were assessed in whole blood samples obtained via a tail nick using a hand-held glucometer and One-Touch glucometer strips (LifeScan Canada, Ltd., Burnaby, British Columbia, Canada). Tail blood samples were taken at baseline and at specific time intervals after drug administration (see *Results*).

Echocardiography

Nonfasted mice were more lightly anesthetized with ketamine-HCl (70 mg/kg ip) and xylazine-HCl (7 mg/kg ip) and placed on a warming pad. Two-dimensional and M-mode echocardiography as well as pulsed Doppler analyses were performed by a blinded observer using a Hewlett-Packard 5500 ultrasound device (Hewlett-Packard Co., Palo Alto, CA) and a 12-MHz phased array and 15 MHz Doppler probes as previously described (30). Three to five M-mode recordings of end-systolic and end-diastolic LV internal diameters, and end-diastolic septal and LV posterolateral wall thickness measurements were made at the level of the papillary muscle. Two observers (blinded to genotype) interpreted each mouse study and agreed on a set of three values for each measurement. A single mean was then calculated for each mouse. For each animal, a LV end-diastolic volume (LVEDV) was estimated from the modified single plane area formula {LVEDV = 0.85 (A^2/L); ml} (Ref. 31) and fractional shortening (%FS; Ref. 32) stroke volume (SV; Ref. 29), cardiac output (CO; Refs. 33 and 34), and systemic vascular resistance (SVR; Ref. 35) were derived from established formulae: %FS = 100 (LVIDd − LVIDs)/LVIDd); SV = %FS × LVEDV; CO = SV × HR; SVR = 80 (MAP − LVEDP)/CO).

Cardiac morphology

Animals were anesthetized as described above, perfused via the left ventricle with 2 M KCl to arrest the heart in diastole, and fixed with 4% buffered formalin at physiological pressure. Hearts were post-fixed in formalin, embedded in paraffin, sectioned at 6 μm, and stained with H&E (hematoxylin and eosin). Cardiac morphometry was performed with Scion Image software (Scion Co., Frederick, MD) using digital planimetry of images obtained from midventricular cross sections.

Assessment of c-fos activation in the murine central nervous system (CNS)

The number of c-*fos*-immunoreactive neurons in specific brain regions was quantitatively assessed in both WT and GLP-$1R^{-/-}$ mice as described (21, 36). Briefly, animals were treated with ip insulin or saline and anesthetized as described above, brains were removed immediately and kept in ice-cold 4% paraformaldehyde solution for 4 d, then transferred to a solution containing paraformaldehyde and 10% sucrose before being cut 12 h later using a sliding microtome Leica SM2000R (Leica Microsystems, Richmond Hill, Ontario, Canada). Sections (25-μm-thick) were collected and stored at −30 C in a cold cryoprotecting solution. Brain sections were processed for immunocytochemical detection of Fos using a conventional avidin-biotin-immuno peroxidase method (Vectastain ABC Elite Kit, Vector Laboratories, Inc., Burlingame, CA) as described (21, 36). The Fos antibody (Sigma-Aldrich Corp., Oakville, Ontario, Canada) was used at a 1:50,000 dilution. Brain sections corresponding to the level of the arcuate nucleus and medial parvocellular portion of the paraventricular nucleus (PVN) of the hypothalamus were selected for analyses. For the latter, sections matching the levels from the bregma −0.70 mm to bregma −0.94 mm were picked according to the Mouse Brain Atlas of Franklin and Paxinos (37).

Neurohumoral responses to stress

For assessment of the hormonal counterregulatory response to insulin-induced hypoglycemia, mice were given ip injections of either 2 U/kg human insulin (Novolinge Toronto; Novo Nordisk A/S, Bagsvaerd, Denmark) or saline. Blood was drawn from a tail vein at 0, 15, 30, 60, and 75 min after insulin or saline administration, and blood glucose levels were measured using a blood glucose meter (Glucometer Elite, Bayer Inc., Toronto, Ontario, Canada). Blood glucose levels (millimoles per liter) fell from an average value of 7.3 at time 0 to 2.1 at 75 min after ip insulin for both WT CD-1 and GLP-$1R^{-/-}$ mice (data not shown). After the last blood glucose measurement at 75 min, mice were anesthetized with Somnotol (sodium pentobarbital solution, MTC Pharmaceuticals, Cambridge, Ontario, Canada) and exsanguinated via cardiac puncture. Blood samples were immediately mixed with a 10% volume of a chilled solution containing 5000 KIU/ml Trasylol (Miles Pharmaceuticals, Etobicoke, Ontario, Canada), 32 mM EDTA, and 0.1 nM diprotin A (Sigma, St. Louis, MO). Plasma was separated by centrifugation at 4 C and stored at −80 C until assayed. Plasma glucagon concentrations were measured by RIA (Linco Research, Inc., St. Charles, MO), and plasma (adrenaline) epinephrine levels were assayed by ELISA (IBL Immuno-Biological Laboratories, Hamburg, Germany).

Data analysis

Unless otherwise noted, all data are expressed as mean ± SE. Statistical analysis was performed using two-way ANOVA or Student's *t* test

where appropriate. Significance was considered when P value was less than 0.05.

Results

Baseline cardiac function

Two-month-old male GLP-1R$^{-/-}$ mice had significantly lower BW compared with age-matched male CD-1 WT controls [29.0 ± 1.1 g (n = 14) *vs.* 33.0 ± 0.5 g (n = 12); $P < 0.003$], consistent with previous observations (38). Although 2-month-old GLP-1R$^{-/-}$ mice displayed a reduced resting HR [223 ± 20 min^{-1} (n = 6) *vs.* 315 ± 33 min^{-1} (n = 6); $P < 0.05$] and elevated LV end-diastolic pressure [LVEDP; 14.5 ± 1.3 mm Hg (n = 6) *vs.* 6.8 ± 1.4 mm Hg (n = 5); $P < 0.01$], no significant differences were observed in aortic SBP and DBP, peak right ventricular systolic pressure, right ventricular end-diastolic pressure, or the maximum rates of LV pressure rise and fall (peak +dP/dT and −dP/dT; Fig. 1).

Five-month-old male GLP-1R$^{-/-}$ mice remained significantly lower in BW than age-matched male CD-1 controls [32.8 ± 0.6 g (n = 22) *vs.* 37.9 ± 0.8 g (n = 22); $P < 0.001$]; however, baseline HR, SBP, DBP, peak LV systolic pressure (LVSP), LVEDP, and peak +dP/dT and −dP/dT were not significantly different (Table 1). Furthermore, echocardiography and Doppler-defined (noninvasive) measures of car-

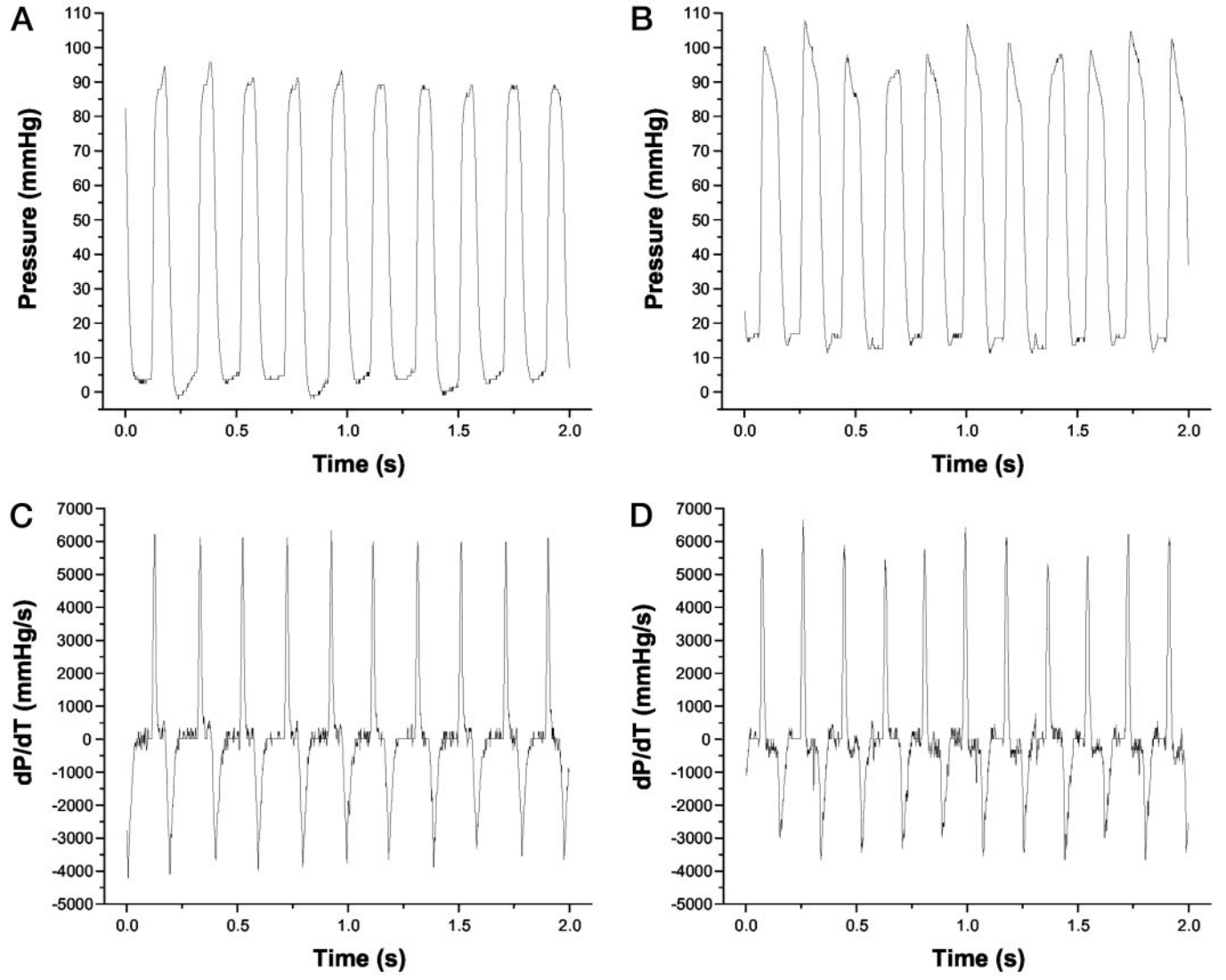

FIG. 1. Hemodynamic monitoring in 2-month-old mice. Representative Millar Mikro-tip pressure-transducing catheter recordings from the LV cavities of anesthetized 2-month-old male CD-1 WT (A) and GLP-1R$^{-/-}$ (B) mice with similar HR (300 min^{-1}) are shown. The first derivatives of the pressure tracings of the WT (C) and GLP-1R$^{-/-}$ (D) mice are also shown. Peak positive (+dP/dT) and negative (−dP/dT) deflections of the first derivate plots are used as measures of contractility and lusitropy, respectively. In this series of investigations (n ≥ 5 for each group), the aortic SBP (101 ± 7 *vs.* 100 ± 7 mm Hg; $P = 0.93$) and DBP (74 ± 6 *vs.* 68 ± 8 mm Hg; $P = 0.53$), peak LVSP (99 ± 5 *vs.* 100 ± 3 mm Hg; $P = 0.85$), peak +dP/dT (7760 ± 1100 *vs.* 5983 ± 361 mm Hg/sec; $P = 0.16$) and peak −dP/dT (5120 ± 963 *vs.* 3367 ± 339 mm Hg/sec; $P = 0.12$) were not significantly different between WT and GLP-1R$^{-/-}$ mice. However, as a group, the 2-month-old GLP-1R$^{-/-}$ animals had significantly lower HR and elevated LVEDP compared with WT controls (see *Results*). In a separate series of experiments (n ≥ 6 for each group), right ventricular systolic pressure (26.3 ± 3.2 *vs.* 25.6 ± 2.3 mm Hg, $P = 0.84$) and right ventricular end-diastolic pressure were not significantly different between WT and GLP-1R$^{-/-}$ mice (1.7 ± 3.7 *vs.* 2.6 ± 1.6 mm Hg; $P = 0.61$).

TABLE 1. Baseline hemodynamic parameters in anesthetized 5-month-old animals

Parameter	WT	GLP-1R$^{-/-}$	*P*
HR (min^{-1})	219 ± 10	224 ± 18	0.81
SBP (mm Hg)	94 ± 5	89 ± 4	0.44
DBP (mm Hg)	62 ± 4	59 ± 3	0.55
LVSP (mm Hg)	96 ± 5	92 ± 4	0.50
LVEDP (mm Hg)	9 ± 1	12 ± 1	0.16
+dP/dT (mm Hg/sec)	4867 ± 538	4621 ± 538	0.74
−dP/dT (mm Hg/sec)	3862 ± 412	3494 ± 396	0.53
CO (ml/min)	3.09 ± 0.31	2.80 ± 0.15	0.38
SVR (mdyne·s/cm^5)	1770 ± 192	1645 ± 77	0.52

Data represent mean ± SEM for n = 13 mice. WT, Age-matched male wild-type CD-1 control mice; GLP-1R$^{-/-}$, age-matched male GLP-1R knockout mice; +dP/dT, maximum positive first derivative of left ventricle pressure; −dP/dT, maximum negative first derivative of left ventricle pressure; CO, cardiac output; SVR, systemic vascular resistance. *P* values represent the results of unpaired Student's *t* test.

diac performance such as fractional shortening, peak aortic flow velocity, cardiac output, and systemic vascular resistance were not significantly different between lightly anesthetized male 5-month-old GLP-1R$^{-/-}$ and CD-1 WT controls (Table 1 and data not shown). Taken together, these data suggest either an age-dependent compensation for the specific baseline cardiac functional abnormalities (resting HR and LVEDP) noted in 2-month-old GLP-1R$^{-/-}$ mice, or differences in the rate of normal cardiovascular development between GLP-1R$^{-/-}$ mice and controls.

Cardiac structure

To ascertain whether GLP-1R$^{-/-}$ mice exhibited changes in cardiac structure, we performed two-dimensional and M-mode echocardiography in 5-month-old lightly anesthetized male GLP-1R$^{-/-}$ mice and WT CD-1 controls. Although absolute septal thickness (ST) and posterolateral myocardial wall thickness (PLT) of GLP-1R$^{-/-}$ and WT mice did not differ [ST: GLP-1R$^{-/-}$, 0.769 ± 0.028 mm (n = 11) *vs.* WT, 0.728 ± 0.038 mm (n = 10); $P = 0.39$; PLT: GLP-1R$^{-/-}$, 0.662 ± 0.016 mm (n = 11) *vs.* WT, 0.658 ± 0.031 mm (n = 10); $P = 0.89$], BW-adjusted values were significantly increased in GLP-1R$^{-/-}$ compared with age-matched control mice (Fig. 2A).

Morphometry of perfusion-fixed hearts from 2- and 5-month-old GLP-1R$^{-/-}$ and WT mice demonstrated significant differences in cardiac dimension. Although the wet heart weight (HW) of 2-month-old GLP-1R$^{-/-}$ mice appeared lower than that of age-matched WT controls [150 ± 11 mg (n = 8) *vs.* 178 ± 10 mg (n = 6); $P = 0.06$], HW/BW ratios were nearly identical [5.26 ± 0.35 mg/g (n = 8) *vs.* 5.28 ± 0.33 mg/g (n = 6); *P* = not significant). By contrast, wet HW was significantly reduced in GLP-1R$^{-/-}$ *vs.* WT controls at 5 months of age [207.3 ± 21.5 mg (n = 6) *vs.* 353.3 ± 26.8 mg (n = 7); $P < 0.002$], and this difference remained significant after correction for BW (Fig. 2B).

Although morphometry of perfusion-fixed cardiac sections also suggested that GLP-1R$^{-/-}$ hearts are smaller than WT hearts at 2 months of age [0.110 ± 0.008 cm^2 (n = 9) *vs.* 0.140 ± 0.012 cm^2 (n = 6); $P = 0.05$], this difference was again far more significant at 5 months of age [0.148 ± 0.025 cm^2 (n = 6) *vs.* 0.240 ± 0.015 cm^2 (n = 7); $P < 0.01$; Fig. 2C].

Quantitative morphometry of histological sections revealed that BW-adjusted ST and PLT wall thickness was significantly increased in older GLP-1R$^{-/-}$ mice [ST/BW, 0.030 ± 0.005 mm/g (n = 5) *vs.* 0.019 ± 0.002 mm/g (n = 7); $P < 0.05$; PLT/BW, 0.032 ± 0.004 mm/g (n = 5) *vs.* 0.021 ± 0.002 mm/g (n = 7); $P < 0.02$], consistent with the results of *in vivo* echocardiography. These data suggest that although baseline cardiac functional abnormalities are no longer detectable in 5-month-old GLP-1R$^{-/-}$ mice, significant differences in cardiac dimension remain detectable in GLP-1R$^{-/-}$ *vs.* WT mice.

Light microscopy did not reveal overt histological differences between H&E-stained cardiac sections of GLP-1R$^{-/-}$ and WT mice at 2 or 5 months of age (Fig. 2, C and D). Specifically, no valvular abnormalities, myofibrillar disarray, necrosis, myocyte loss, inflammatory infiltration, or increased matrix deposition could be identified as possible explanations for either the decreased heart size or increased wall thickness of GLP-1R$^{-/-}$ mice (Fig. 2, C and D).

Cardiac responses to stress hormones

To assess whether the cardiovascular consequences of GLP-1R gene disruption might be unmasked by stress hormone-mediated activation of cardiac function, we examined the impact of 1) insulin and 2) adrenaline on cardiovascular parameters in both CD-1 WT and GLP-1R$^{-/-}$ mice at 5 months of age. Insulin administration (at time 0) produced a nonsignificant reduction in blood glucose (millimoles per liter) in anesthetized mice over the duration of the experiment (10 min; WT, 8.38 ± 0.30 *vs.* 7.34 ± 0.37, $P = 0.061$; GLP-1R$^{-/-}$, 8.98 ± 0.34 *vs.* 8.06 ± 0.39, $P = 0.113$; initial *vs.* final blood glucose level, Fig. 3A). Both WT and GLP-1R$^{-/-}$ mice exhibited significant increases in HR in response to insulin that did not differ significantly between WT and GLP-1R$^{-/-}$ mice (Fig. 3B). By contrast, significant differences in LVSP, LVEDP, and contractility (peak +dP/dT) were noted in WT *vs.* GLP-1R$^{-/-}$ mice after insulin administration (Fig. 3, C–E). Insulin administration was associated with a transient increase in both LV contractility (Fig. 3E) and lusitropy (Fig. 3F) in WT but not GLP-1R$^{-/-}$ mice. In contrast, the significant elevations of LVEDP noted in insulin-treated GLP-1R$^{-/-}$ but not WT mice were sustained for the entire duration of monitoring (Fig. 3D) and did not correct even after other hemodynamic parameters appeared to have normalized.

To determine whether the defective cardiovascular responses observed in stressed GLP-1R$^{-/-}$ mice were attributable to primary cardiac abnormalities or to potential defects in central GLP-1R-mediated activation of the neurohormonal stress response, we examined both central and peripheral responses to a metabolic stress in WT and GLP-1R$^{-/-}$ mice. Activation of c-*fos* expression in the glucose-sensitive PVN was significantly increased after insulin administration in both WT and GLP-1R$^{-/-}$ mice (Fig. 4, A and B). Insulin-induced hypoglycemia was also associated with significant induction of c-*fos* expression in the arcuate nucleus of both WT and GLP-1R$^{-/-}$ mice (data not shown). To determine whether insulin-induced hypoglycemia was associated with appropriately increased levels

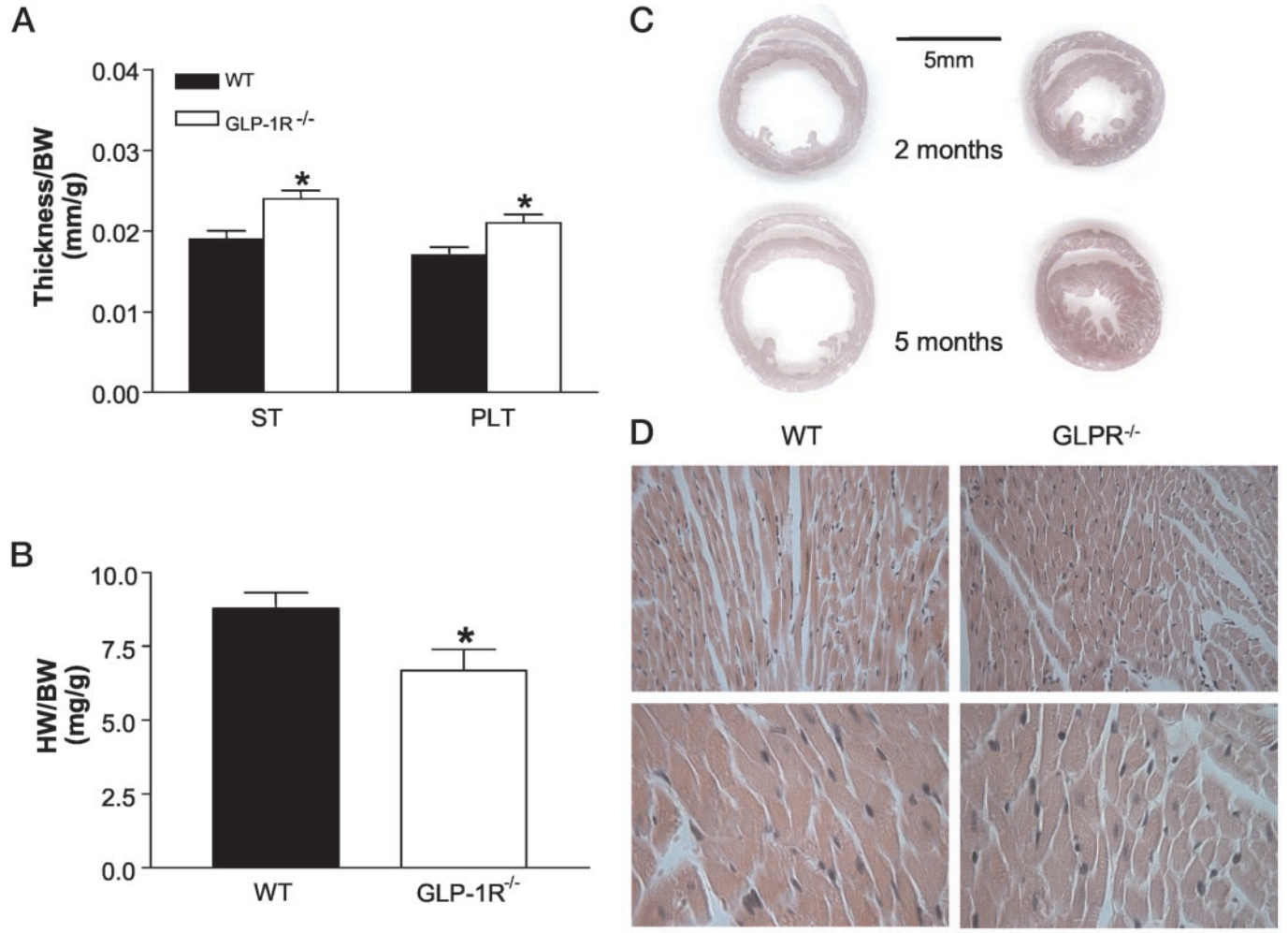

FIG. 2. Cardiac morphometry and histology. Data shown in Panels A-B were obtained from male 5 month-old GLP-1R$^{-/-}$ and CD-1 WT mice. A, Weight-adjusted baseline M-mode echocardiography-derived ST and PLT wall thickness in lightly anesthetized animals. B, The wet HW/BW ratio. Data shown are the mean ± SEM. *, $P < 0.05$ via unpaired Student's *t* test. C, Representative midventricular cardiac sections of 2- and 5-month old GLP-1R$^{-/-}$ and CD-1 WT mice. *Scale bar*, 5 mm. D, Representative photomicrographs of H&E-stained histological sections. Magnification, *Top*, ×40; *bottom*, ×100 oil immersion.

of counterregulatory hormones, plasma glucagon and adrenaline were measured after insulin administration. Baseline levels of adrenaline and glucagon were comparable and increased significantly in response to hypoglycemia in both WT and GLP-1R$^{-/-}$ mice (Fig. 4, C and D). These data indicate that GLP-1R$^{-/-}$ mice are capable of sensing and responding appropriately to metabolic stress, suggesting that the abnormal cardiac responses to insulin observed in GLP-1R$^{-/-}$ mice were not likely attributable to defects in the central or peripheral arms of the neurohormonal stress response.

To ascertain further whether the defective cardiovascular responses were secondary to primary defects in cardiac function, we examined the cardiac responses to adrenergic stimulation in both WT and GLP-1R$^{-/-}$ mice at 5 months of age. Adrenaline (epinephrine) administration (at time 0) produced a nonsignificant increase in blood glucose levels (millimoles per liter) in anesthetized mice over the duration of the 10-min experiment (WT, 10.60 ± 0.81 *vs.* 12.63 ± 0.89, $P = 0.143$; GLP-1R$^{-/-}$, 9.82 ± 0.68 *vs.* 11.10 ± 0.99, $P = 0.318$; for initial *vs.* final glucose level, Fig. 5A). Adrenaline administration was associated with a rapid (1 min) but transient increase in HR in both WT and GLP-1R$^{-/-}$ mice, with no significant difference between the HR responses of WT *vs.* GLP-1R$^{-/-}$ mice (Fig. 5B). Adrenaline rapidly but transiently increased peak LVSP (Fig. 5C) and decreased LVEDP (Fig. 5D) in both WT and GLP-1R$^{-/-}$ mice. However, the LVSP response of GLP-1R$^{-/-}$ mice was significantly less than that of WT mice (Fig. 5C). Similarly, adrenaline-induced increases in LV contractility (peak +dP/dT) were significantly more transient in GLP-1R$^{-/-}$ compared with WT mice (Fig. 5E). Although the lusitropic response to adrenaline appeared to be blunted in GLP-1R$^{-/-}$ mice compared with WT controls (peak −dP/dT; Fig. 5F), the difference between the GLP-1R$^{-/-}$ and WT responses was not statistically significant. Of note, a similar result was observed in the lusitropic response to insulin (Fig. 3F).

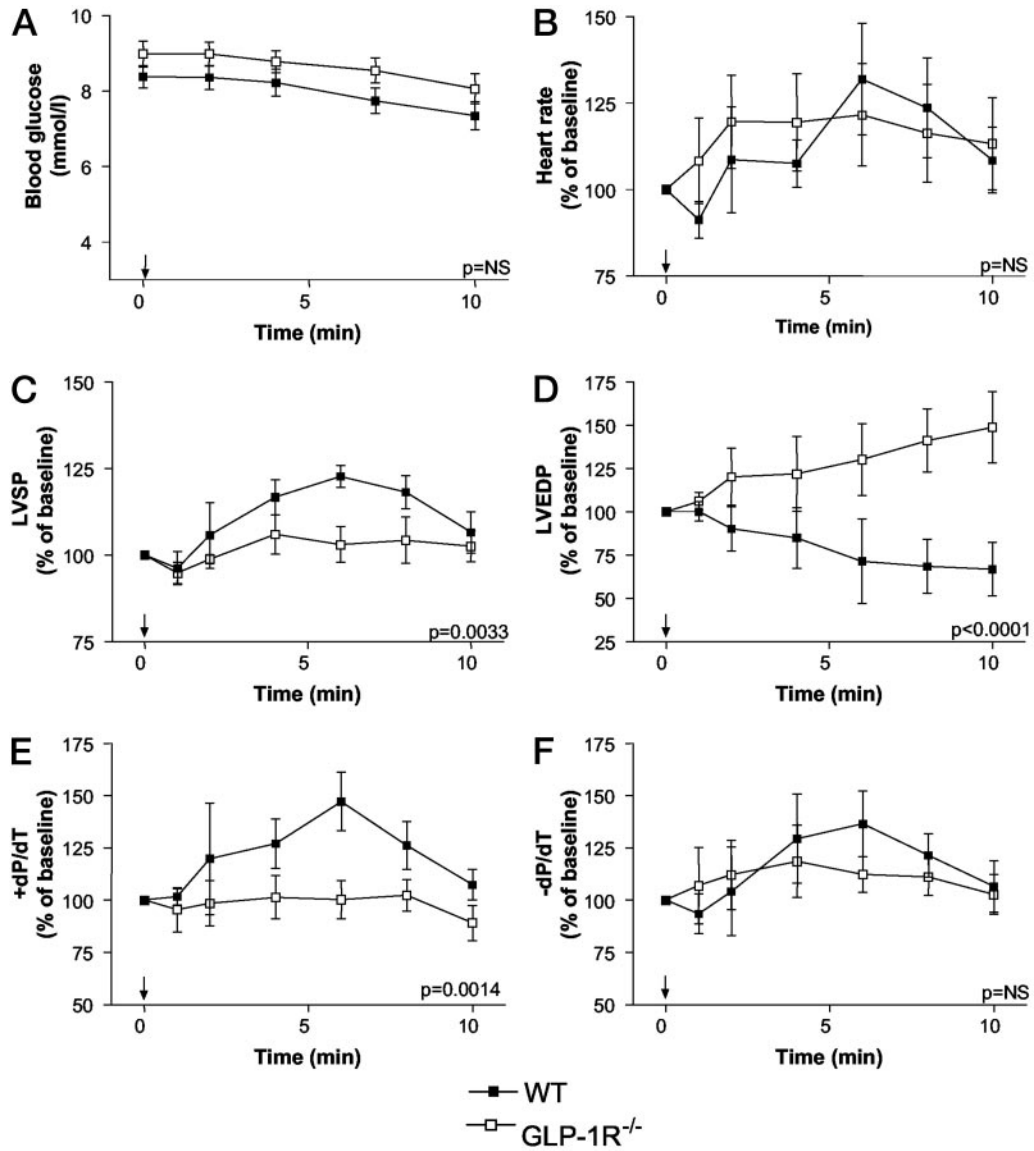

FIG. 3. Cardiac hemodynamic responses to insulin in 5-month-old mice. Effect of insulin (3 U/kg, ip, administered at *arrow*) on blood glucose (A), HR (B), LVSP (C), LVEDP (D), +dP/dT (E), and −dP/dT (F) in 5-month-old WT (*closed symbols*) and GLP-1R$^{-/-}$ (*open symbols*) mice. Data for B, C, D, E, and F are expressed as percentage of baseline (time = 0) parameters. Statistical analyses between WT and GLP-1R$^{-/-}$ curves were by two-way ANOVA, with n = 5 for each group.

Discussion

Although cardiac expression of the GLP-1R has been identified in humans (23) and rodents (10), a direct role for cardiac GLP-1R signaling *in vivo* had not been demonstrated previously. Using isolated rat cardiac myocytes *in vitro*, Vila Petroff *et al.* (22) showed that GLP-1R signaling results in an increase in levels of intracellular Ca^{2+} and cytosolic cAMP, which did not induce any appreciable effects on contractile responses. Several studies have demonstrated that GLP-1 or exendin-4 infusions resulted in significant increases in both HR and BP in rats (20, 26). However, these effects appeared dependent in part on the CNS and vagal innervation (27), and exogenous administration of GLP-1R agonists also activated CNS regions involved in cardiac autonomic regulation (21). Accordingly, the relative contributions to cardiovascular physiology of the responses of the CNS and peripheral nervous system to GLP-1, *vs.* more direct effects on cardiac GLP-1 receptors *in vivo*, remained uncertain.

The new data presented here demonstrate that GLP-1R$^{-/-}$ mice exhibit significant differences in cardiac function compared with WT CD-1 controls, strongly suggesting that their distinctly blunted cardiac responses are at least partly due to cardiac defects and not strictly attributable to indirect central or peripheral neurohumoral mechanisms.

Hemodynamic abnormalities in GLP-1R$^{-/-}$ mice

The reduced HR of 2-month-old GLP-1R$^{-/-}$ mice is consistent with the known positive chronotropic effect of GLP-1 in rodents (Refs. 20, 21, 26, and 27; see below). Given that the baseline SBP and DBP and peak LVSP of 2-month-old GLP-

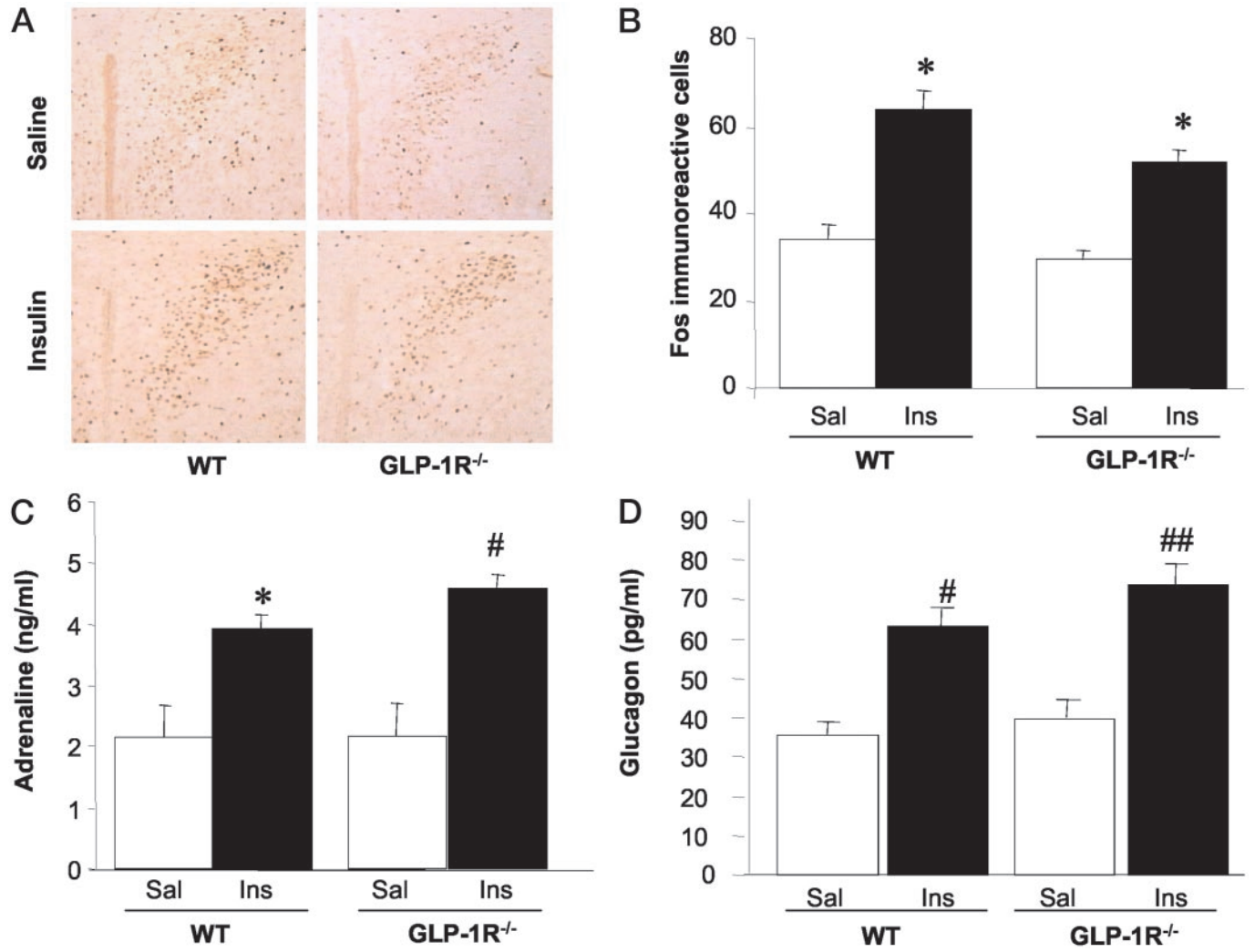

FIG. 4. Central and peripheral neurohumoral responses to insulin. Assessment of central hypothalamic c-*fos* expression and counterregulatory hormonal responses to insulin-induced hypoglycemia were performed as described in *Materials and Methods*. A, Representative photomicrographs of Fos-stained coronal brain sections of the PVN of the hypothalamus illustrate the effects of insulin-induced hypoglycemia on the number of Fos-immunopositive cells in CD-1 WT and GLP-1R$^{-/-}$ mice. Magnification, ×100. B, The mean (±SE) numbers of Fos-immunoreactive cells counted in the PVN. Five brain sections were analyzed per mouse, with n = 7–10 mice in each group. *, $P < 0.05$ for saline- *vs.* insulin-injected mice. C and D, Mean (±SE) plasma adrenaline (C) and glucagon (D) levels in mice after the administration of saline or insulin are shown, with n = 4–8 mice/group. *, $P < 0.05$; #, $P < 0.02$; ##, $P < 0.01$ for saline- *vs.* insulin-injected mice by ANOVA.

1R$^{-/-}$ mice were no different from those of WT animals (Fig. 1), it is unlikely that the observed difference in HR can be attributed to an increased sensitivity to the weight-adjusted anesthetic. Rather, it suggests that an intact GLP-1R in young mice may have a more important role in cardiac pacemaker function than on ventricular contractility.

Importantly, the reduced HR of 2-month-old GLP-1R$^{-/-}$ mice cannot explain the significant elevation in their LVEDP. Indeed, prolongation of diastole is expected to improve and not exacerbate measures of diastolic performance. In the absence of significant defects in active lusitropy (peak −dP/dT), elevations in LVEDP may still be explained by abnormalities in the active and/or passive phases of diastole, or an increase in the effective circulating blood volume. Given their reduced BW and the absence of any signs of pulmonary congestion, edema, or an increase in right ventricular pressures or systemic blood pressure, the latter possibility is unlikely. Rather, a direct defect in diastolic function would be consistent with the small, thickened, LV cavity documented in older GLP-1R$^{-/-}$ animals (Fig. 2) and the significant abnormalities in LVEDP and dP/dT noted after stress hormone administration (Figs. 3 and 5). Although, not statistically significant ($P = 0.16$), a small but potentially functionally significant increase in LVEDP remained evident in 5-month-old GLP-1R$^{-/-}$ mice (Table 1). Future studies exploring *in vivo* and/or *ex vivo* pressure-volume relationships will be required to dissect further the mechanisms underlying this abnormality.

Structural abnormalities in GLP-1R$^{-/-}$ mice

Notwithstanding their significantly reduced BW, the lower HW/BW ratio and smaller planimetry-defined cross-sectional area of perfusion-fixed GLP-1R$^{-/-}$ hearts suggest

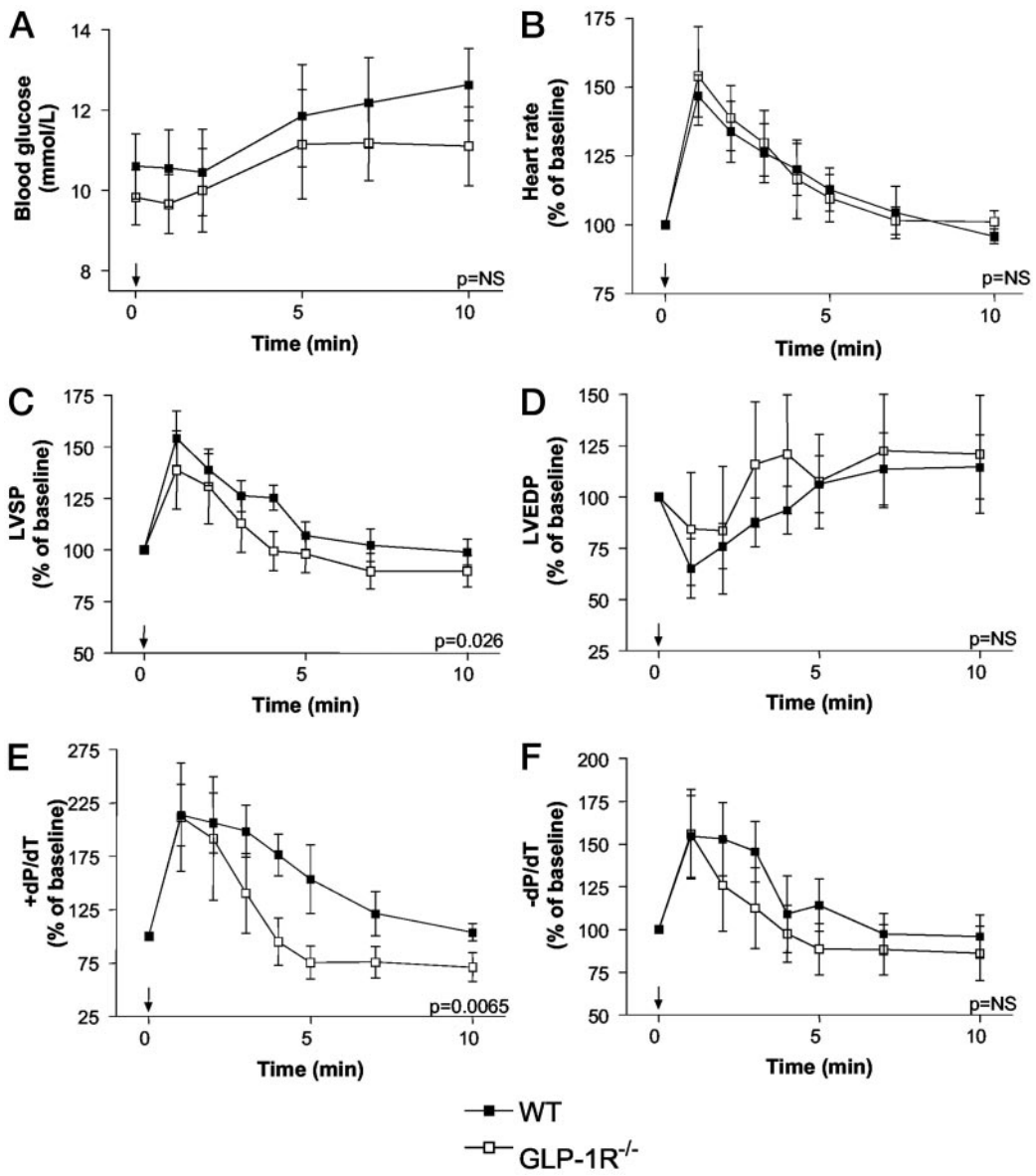

FIG. 5. Cardiac hemodynamic responses to adrenaline in 5-month-old mice. Effect of adrenaline (1 μg/kg, iv, administered at *arrow*) on blood glucose (A), HR (B), LVSP (C), LVEDP (D), +dP/dT (E), and −dP/dT (F) in 5-month-old WT (*closed symbols*) and GLP-1R$^{-/-}$ (*open symbols*) mice. Data in B, C, D, E, and F are expressed as percentage of the baseline (time = 0) parameter. Statistical analyses between WT and GLP-1R$^{-/-}$ curves were by two-way ANOVA, with n = 6 for each group.

a potential role for GLP-1R in cardiac growth. Consistent with these findings, GLP-1R agonists exert proliferative and cytoprotective effects in the endocrine pancreas (39), and GLP-1R$^{-/-}$ mice exhibit modest but significant perturbations in the number and size of pancreatic islets (40). Interestingly, after correcting for BW, GLP-1R$^{-/-}$ mice appear to have thicker ventricular walls than expected. Given the absence of an increased BP (afterload) or larger cardiac diameter, the increase in LV wall thickness noted in GLP-1R$^{-/-}$ mice may represent an adaptive response to an isolated elevation in diastolic wall tension (see above). If so, this would represent a rather unique model of compensatory mural hypertrophy, deserving of further investigation.

Cardiac effects of glucagon and GLP-1

Several lines of evidence support a role for glucagon-like peptides in cardiac physiology. Both glucagon and GLP-1 exert overlapping physiological actions in different organs, including regulation of appetite (41–43), control of renal function (44, 45), and modulation of gastrointestinal motility (46–49). The glucagon receptor is expressed in the heart (50, 51), and exogenous glucagon administration produces positive chronotropic and inotropic responses in rodents and human subjects (52, 53). These actions have led to the occasional use of glucagon as an agent for the treatment of refractory bradycardia or hypotension (54, 55). Glucagon exerts direct effects in the heart by modulation of cardiac Ca^{2+}

current, activation of adenylyl cyclase, and inhibition of phosphodiesterase (56). Intriguingly, a smaller peptide derived from glucagon via proteolytic cleavage, glucagon (19–29), also regulates myocardial Ca^{2+} flux and amplifies the inotropic effects of glucagon on the heart via effects on cardiac phosphodiesterase activity (57). Whether glucagon receptor signaling is essential for cardiovascular function remains unclear, because parameters of cardiac physiology have not been reported in mice with genetic inactivation of the glucagon receptor (58).

Although less extensively studied, GLP-1 administration also produces dose-dependent increases in HR and BP in anesthetized rats after administration of the native peptide GLP-1(7–36)amide (20). Similar but less robust findings were observed after infusion of glucagon, whereas no changes in HR or BP were detected after administration of GLP-2 (20), consistent with the absence of detectable GLP-2R mRNA transcripts in the heart (12). The cardiovascular effects of GLP-1 were not abolished by pretreatment of rats with reserpine, propranolol, or phentolamine, suggesting that GLP-1 actions on the heart are not strictly dependent on catecholaminergic pathways (20). The cardiac actions of GLP-1 in anesthetized rats were mimicked but more prolonged after administration of the lizard GLP-1 agonist exendin-4, and they were blocked by the GLP-1R antagonist exendin(9–39) (Ref. 26). Taken together, these data are consistent with a role for the known GLP-1R in the transduction of GLP-1-mediated cardiovascular effects.

Evidence implicating both direct and indirect cardiac effects of GLP-1

The importance of both central and peripheral inputs for GLP-1 action on the cardiovascular system is exemplified by several studies. Intracerebroventricular (icv) or iv administration of GLP-1R agonists produced increases in HR and BP in rats that were blocked by icv administration of the GLP-1R antagonist exendin(9–39) (Ref. 27). The importance of neural innervation for cardiovascular GLP-1 actions is further illustrated by the demonstration that bilateral vagotomy blocked the increase in HR and BP induced by central GLP-1 and abolished the cardiovascular effects of central exendin(9–39) on icv, but not peripheral, GLP-1 (27). These findings suggest that the cardiovascular actions of GLP-1 reflect integration of both indirect neural and direct cardiac actions of GLP-1R signaling.

Additional evidence for the importance of central GLP-1 effects on cardiovascular function derives from experiments with nonanesthetized rats administered native GLP-1 or exendin-4 at low doses so as to avoid confounding hypoglycemia or hyperinsulinemia (21). Administration of GLP-1R agonists to normoglycemic rats dose-dependently increased BP and HR and induced c-*fos* expression in the adrenal medulla and neurons in the hypothalamus and brain stem (21). These neurons included medullary catecholamine neurons that provide input to sympathetic preganglionic neurons. Furthermore, GLP-1R agonists rapidly activated tyrosine hydroxylase gene transcription in brain stem catecholamine neurons, providing a link between GLP-1R signaling and activation of the sympathetic nervous system in the nonanesthetized rodent *in vivo* (21).

Importance of GLP-1R in cardiac physiology

Acute iv or icv administration of the GLP-1R antagonist exendin(9–39) had no effect on basal HR or BP in anesthetized rats (26, 27). Similarly, we did not observe significant

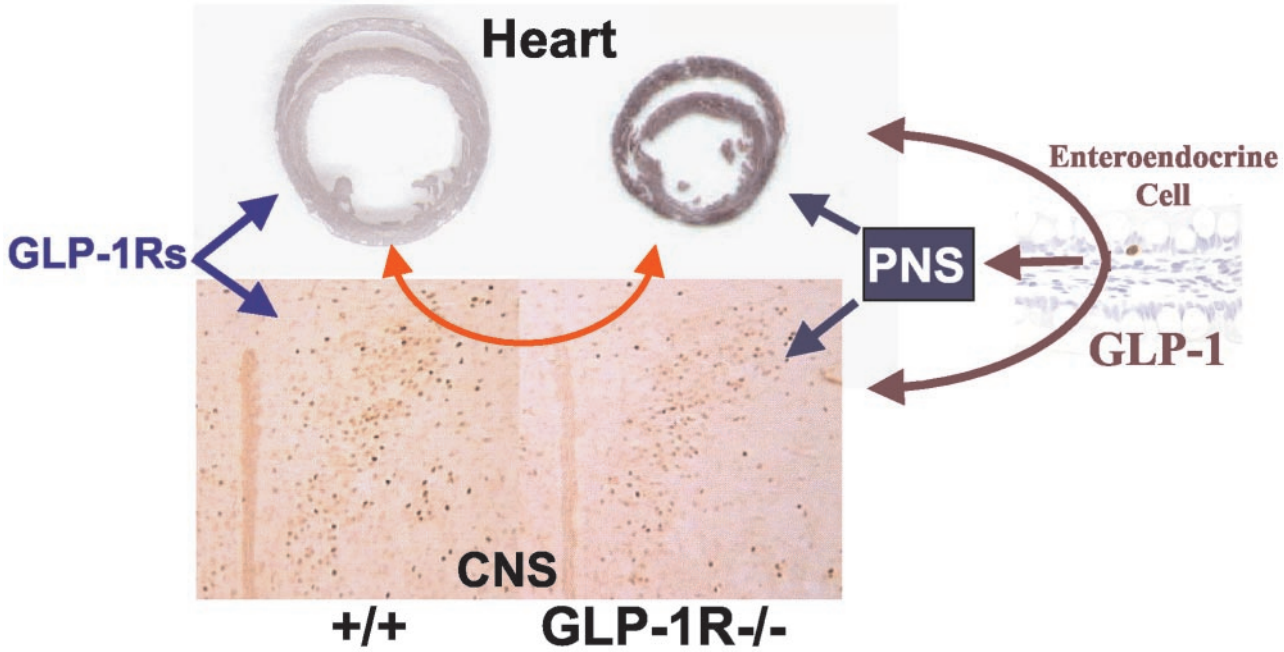

FIG. 6. Schematic depiction of GLP-1-dependent regulation of cardiac function. The proposed model involves GLP-1, secreted from gut endocrine cells, acting either directly on heart GLP-1Rs, or indirectly via the peripheral nervous system (PNS) and/or CNS to regulate cardiovascular function. *Top*, Representative hearts from +/+ WT and GLP-1R$^{-/-}$ mice; *bottom*, fos-positive neurons in the PVN.

changes in these parameters at baseline in 5-month-old GLP-$1R^{-/-}$ mice. In contrast, the cardiac responses to insulin administration were significantly blunted in GLP-$1R^{-/-}$ mice. Furthermore, the defective cardiovascular responses were not attributable to differences in blood glucose. Moreover, despite evidence implicating the GLP-1R in the CNS response to aversive stress (59, 60), the cardiovascular defect was not secondary to problems with sensing or responding to metabolic stress, because activation of counterregulatory stress-responsive pathways, as assessed by central c-*fos* expression and peripheral plasma glucagon and epinephrine levels, were comparable in WT and GLP-$1R^{-/-}$ mice.

Instead, data demonstrating abnormalities in cardiac contractility, HW, wall thickness, and area strongly implicate a direct role for GLP-1R signaling in formation of normal cardiac structure in the developing and/or adult heart (Fig. 6). Activation of GLP-1R signaling in rat cardiomyocytes produced increases in cAMP formation that were blocked by exendin(9–39) (Ref. 22). Although both GLP-1 and isoproterenol produced an intracellular acidosis, GLP-1, but not isoproterenol, induced a decrease in contraction amplitude with no effect on intracellular Ca^{2+} transit (22). Taken together, the previous data and our present studies demonstrating structural and functional cardiac abnormalities in the GLP-$1R^{-/-}$ mouse suggest that the role of GLP-1 in cardiovascular physiology is complex, involves both direct and indirect effects on the nervous system and heart (Fig. 6), and merits further investigation.

Implications for the clinical use of GLP-1R agonists

The implications of our findings in mice for the use of agents activating GLP-1R signaling for the treatment of human diabetes remain uncertain. Continuous infusion of GLP-1 for 48 h produced no major effect on HR, but SBP and DBP tended to be lower in GLP-1-treated patients (61). Subcutaneous administration of native GLP-1 for 6 wk in 20 human subjects with type 2 diabetes was not associated with abnormalities in HR or BP regulation (62). Hence, the role of GLP-1R signaling in the developing or adult rodent cardiovascular system may differ from the role(s) played by the GLP-1R in the human heart. Nevertheless, the GLP-1R is expressed at high levels in the human heart (23), and the long-term cardiovascular effects of more potent degradation-resistant GLP-1 agonists (currently in clinical trials for the treatment of type 2 diabetes) are not yet known. The findings that GLP-1R activation produces increases in HR, BP, and sympathetic nervous system activation in rodents (20, 26, 27), taken together with our data demonstrating structural and functional abnormalities in GLP-$1R^{-/-}$ mice, suggest that ongoing scrutiny of the cardiovascular effects of GLP-1R agonists appears prudent.

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