Cellular Mechanisms Responsible for the Inotropic Action of Insulin on Failing Human Myocardium

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Background: An increase in intracellular calcium transients is responsible for the positive inotropic effect of insulin on human myocardium, but the mechanisms involved in this increase in $[Ca^{2+}]_i$ remain unclear.

Methods: We studied isolated trabeculae or cardiomyocytes from end-stage failing hearts of 38 patients undergoing heart transplantation. The effect of insulin on isometric twitch force (37°C, 0.5 Hz) and L-type Ca^{2+} current (whole-cell voltage clamp) was assessed.

Results: Crystalline insulin increased the contractile force in a dose-dependent manner (0.01 to 10 μmol/liter), with a maximum increase of 45 ± 8% ($p < 0.05$) at 1 μmol/liter. It also increased L-type Ca^{2+} peak current density by 26 ± 6% ($p < 0.05$). This insulin-mediated positive inotropic effect was unchanged in the presence of propranolol (1 μmol/liter). Positive inotropy was partially independent of glucose. L-type Ca^{2+} channel blockade (diltiazem, 5 μmol/liter), and sarcoplasmic reticulum (SR) Ca^{2+}-release channel blockade (ryanodine, 0.1 μmol/liter) did not affect the inotropic response to insulin. However, blockade of SR Ca^{2+}-ATPase (cyclopiazonic acid, 10 μmol/liter), inhibition of Na^+-H^+ exchange (HOE642, 10 μmol/liter), and inhibition of Na^+-Ca^{2+} exchange (SEA0400, 1 μmol/liter) partially prevented the inotropic response to insulin.

Conclusions: Positive inotropy of insulin was not related to catecholamine release and subsequent stimulation of β-adrenergic receptor, but it may enhance the activity of SR Ca^{2+}-ATPase and trans-sarcolemmal Ca^{2+} entry, mainly via reverse-mode Na^+-Ca^{2+} exchange and insulin-mediated activation of Na^+-H^+ exchange. We hypothesize that these changes in $[Ca^{2+}]_i$ might be secondary to the activation of reverse-mode Na^+-Ca^{2+} exchange, presumably via elevated intracellular Na^+ concentration. J Heart Lung Transplant 2006;25:1126–34. Copyright © 2006 by the International Society for Heart and Lung Transplantation.

The essential hormone, insulin, regulates various physiologic and pathophysiologic functions in the heart. These include myocardial energy metabolism, cardiomyocyte contractility, protein production, hypertrophy and cardiomyopathy in patients with diabetes mellitus (DM), and also ion-transport mechanisms.[1] Chronic heart failure is often associated with insulin resistance.[2] Parsonage et al[3] demonstrated that short-term application of insulin has significant hemodynamic effects, with selective skeletal muscle vasodilation associated with better redistribution of peripheral blood flow and increased cardiac output in patients with this condition. However, insulin exerts endothelium-dependent vasodilatory actions,[4] and it remains unclear whether these improved hemodynamics result from the direct inotropic or peripheral vasodilatory effects of insulin.

In normal mammalian hearts[5–8] and isolated cardiac muscle preparations,[5,9–11] insulin exerts positive inotropic (an increase in contractile force) effects, but its sub-cellular mechanism remains unclear. Because of the well-known action of insulin in promoting entry of glucose into the cell, and subsequent stimulation of the carbohydrate metabolic pathway, it is conceivable that the positive inotropy of insulin may be associated with enhancement of glycolytic flux in the myocardium.[5] However, this concept was not substantiated by previous studies,[5,6,8,10] which provided evidence that the increase

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in contractile force produced by insulin is independent of its action on glucose transport in the myocardium.

Because the calcium ion (Ca^{2+}) is known to play a crucial role in excitation–contraction coupling in cardiac muscle,[12] it is possible that insulin-induced positive inotropy may be mediated through changes in Ca^{2+} movements in the cell. This view is supported by the findings that different membrane systems involved in Ca^{2+} transport and translocation (Na^{+}-Ca^{2+} exchange, sarcoplasmic reticulum [SR] Ca^{2+}-ATPase and L-type Ca^{2+} channels) are affected by insulin.[11,13–16] Recently, von Lewinski et al[11] reported that insulin exerts Ca^{2+}-dependent and Ca^{2+}-independent positive inotropic effects through a phosphatidylinositol-3-kinase (PI3K)-dependent pathway in the failing human myocardium. The increased intracellular Ca^{2+} transient ($[Ca^{2+}]_i$) originates at least in part from enhanced reverse-mode Na^{+}-Ca^{2+} exchange and, consequently, increased Ca^{2+} load in the SR. However, the cellular mechanisms involved in the increase in $[Ca^{2+}]_i$ of insulin in failing human myocardium remain unclear.

In this study, we examined the mechanisms behind the positive inotropic action of insulin in isolated failing human myocardium and ventricular myocytes using various pharmacologic interventions known to modify Ca^{2+} movements in the cardiomyocyte. Our main findings show that insulin-mediated positive inotropy is not related to catecholamine release and the subsequent stimulation of β-adrenergic receptors. This positive inotropy was partially independent of glucose. It could be related to an enhanced activity of SR Ca^{2+}-ATPase, to trans-sarcolemmal Ca^{2+} entry mainly via reverse-mode Na^{+}-Ca^{2+} exchange, and to an insulin-mediated activation of Na^{+}-H^{+} exchange.

Some of the findings in this study have been presented elsewhere in abstract form.[17]

METHODS

Human Ventricular Tissue Preparations

Experiments were performed using ventricular trabeculae obtained from 38 patients in end-stage myocardial failure (mean age 45 ± 4 years; 29 men, 9 women) who underwent heart transplantation at Cheng-Hsin General Hospital and Tri-Service General Hospital. The investigation conformed to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from each patient and the institutional ethics committee approved the protocol. Baseline clinical characteristics of patients are shown in Table 1. Human ventricular trabeculae (approximately 0.5 to 1.5 mm in diameter and 5 to 10 mm long) were dissected from the right (>90%) or left ventricle and perfused with Tyrode's solution with the following composition (in millimoles per liter): NaCl, 137; KCl, 4; $MgCl_2$, 0.5; $NaHCO_3$, 15; NaH_2PO_4, 0.5; $CaCl_2$, 2.7; and dextrose, 5.5. The solution was gassed with a mixture of 97% O_2 and 3% CO_2, yielding a pH of approximately 7.4 at 37°C.

Table 1. Characteristics of Patients Undergoing Heart Transplantation

	Total	DCM	ICM
Number of Patients	38	30	8
DM/non-DM	10/28	6/24	4/4
Age (y)	45 ± 4	45 ± 3	45 ± 5
Peak Vo_2 (ml/kg/min)	12.1 ± 0.5	12.2 ± 0.5	11.9 ± 1.3
LVEF (%)	23.4 ± 0.8	23.2 ± 1.1	25.1 ± 1.6
CI (liters/min/m^2)	1.6 ± 0.1	1.7 ± 0.1	1.4 ± 0.1
PCWP (mm Hg)	27 ± 2	27 ± 2	28 ± 3
Treatment (% of patients)			
Beta-blockers	47%	46%	50%
Digitalis	76%	78%	68%
Diuretics	82%	83%	78%
ACE inhibitors	78%	79%	74%
Inotropes (IV)	38%	38%	38%

DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; DM, diabetes mellitus, fasting blood sugar > 126 mg/dl; Peak Vo_2 peak ventilatory oxygen uptake; LVEF, left ventricular ejection fraction; CI, cardiac index; PCWP, pulmonary capillary wedge pressure; ACE, angiotensin-converting enzyme. Oral medication was terminated at least 12 hours before surgery.

Action Potentials and Contraction Recording

As described elsewhere,[18] one end of the preparation was fixed to the bottom of the tissue bath and the other end was tied by means of a short silk thread to a stainless-steel rod attached to a force-displacement transducer (Model FT03C; Grass Instruments, Quincy, MA). Muscles were perfused with bicarbonate-containing Tyrode's solution and stimulated at 0.5 Hz (37°C). Isometric contractions were recorded at an optimal pre-load (L_{max}) after equilibration for 60 minutes. The action potentials were recorded by means of glass microelectrodes filled with 3 mol/liter KCl and connected to an electrometer (WPI Duo 223; World Precision Instruments, New Haven, CT). Electrical and mechanical events were displayed simultaneously on an oscilloscope (Model 4072; Gould Instruments, Cleveland, OH) and recorder (Model TA11; Gould). The functional effects of insulin were assessed by cumulative concentration–response measures (0.01 to 10 μmol/liter), or by a single, maximally effective concentration of insulin (1 μmol/liter). For experiments in which we tested the effects of exogenous glucose on the action of insulin, glucose was omitted from the Tyrode's solution.

Isolation of Human Ventricular Myocytes

Human ventricular myocytes were isolated by modified enzymatic dissociation,[19] using a Langendorff-type apparatus for coronary artery perfusion. A portion of the ventricle (~6 × 4 cm) was removed, along with a branch of the coronary artery. After cannulation, the myocardium was perfused for 30 minutes with a nominally Ca^{2+}-free HEPES–Tyrode's solution (37°C, pH 7.4). Digestion was performed with the same solution containing 1 mg/ml Type I collagenase (Sigma-Aldrich, St. Louis, MO), 0.1 mg/ml Type XIV protease (Sigma-Aldrich) and 1 mg/ml

bovine serum albumin (Sigma-Aldrich) for 20 to 30 minutes. The enzyme-containing solution was washed from the tissue for 10 minutes with Ca^{2+}-free HEPES–Tyrode's solution. The perfusate was bubbled with 100% O_2 and warmed to 37°C. The specimen was then minced with fine scissors in the same solution. Isolated cells were separated from the minced tissue by gravity filtration through 400-μm mesh filter gauze and then stored in modified Kraft-Brühe medium[20] (KB)[20] and Ca^{2+}-free HEPES–Tyrode's solution at room temperature. Only Ca^{2+}-tolerant, clearly striated, rod-shaped cells without any blebs were studied, with the isolation giving an initial yield of 5% to 20% of total cells.

Patch-clamp Experiments

For measurement of L-type Ca^{2+} currents ($I_{Ca,L}$), a ruptured whole-cell patch-clamp technique[21] was applied using an Axopatch 1D amplifier (Axon Instruments, Union City, CA). Data acquisition and analysis were controlled using pCLAMP software (Axon Instruments). The external solution contained (in millimoles per liter): tetraethylammonium chloride, 137; $MgCl_2$, 0.5, $CaCl_2$, 1.8, CsCl, 5.4; glucose, 11; and HEPES, 10 (adjusted to pH 7.4 with CsOH). The internal solution contained (in millmoles per liter): CsCl, 130, $MgCl_2$, 1.0, NaGTP, 0.1; ethylene-glycol tetraacetic acid (EGTA), 10; Mg_2-ATP, 5; Na_2-phosphocreatine, 5; and HEPES, 10 (adjusted to pH 7.2 with CsOH). Myocytes were placed in a 0.5-ml chamber and perfused with external solution (35 ± 1°C). A small hyperpolarizing step from a holding potential of −50 mV to a testing potential of −55 mV was delivered at the beginning of each experiment.

The area under the capacitative currents was divided by the applied voltage step to obtain the total cell capacitance. It was assumed that the cell membranes had a specific membrane capacitance of 1 μF/cm^2. $I_{Ca,L}$ was measured at a rate of 0.1 Hz using depolarizing voltage pulses of 300 milliseconds clamped from the holding potential of −50 mV to test potentials increasing from −40 mV up to +60 mV in 10-mV steps. $I_{Ca,L}$ was measured as the difference between the peak inward current and the average current during the last 10 milliseconds of the 300-millisecond pulse. At steady-state basal current recordings, insulin (1 μmol/liter) was added to the perfusate and the effects of insulin on L-type Ca^{2+} currents were analyzed. The time-course of inactivation of $I_{Ca,L}$ was also examined.[22] From peak current to end-pulse current, traces were fitted by double-exponential functions (Clampfit, Axon Instruments) of the form:

$$I = A_0 + A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$$

where I is the current at Time t, A_1 and A_2 are the amplitudes of the current at Time 0 of the individual components, and τ_1 and τ_2 are their respective time constants. A_0 is a constant that represents the current at Time = ∞.

Drugs

Crystalline recombinant human insulin (Sigma-Aldrich) stock aliquot was diluted in Tyrode's solution and added to the tissue bath. Diltiazem hydrochloride, propranolol hydrochloride, ryanodine, cyclopiazonic acid (all from Sigma-Aldrich), HOE642 (Cariporide; a gift of Sanofi-Aventis Pharma, Frankfurt, Germany) and SEA0400 (a gift from Taisho Pharmaceutical Co., Saitama, Japan) were added to the tissue bath at least 30 minutes before the experiment.

Statistical Analyses

Values are presented as mean ± SEM. Differences were compared using paired Student's *t*-tests or 1-way repeated-measures analysis of variance (ANOVA), followed by Student–Newman–Keuls tests when appropriate. $p < 0.05$ was considered statistically significant.

RESULTS

Inotropic Effects of Insulin

The concentration–response data of insulin for six preparations are summarized in Figure 1. It is clear that insulin exerted positive inotropy, starting from a concentration of 0.01 μmol/liter, and reached a peak inotropy (45 ± 8%, $p < 0.05$) at 1 μmol/liter. As shown in Figure 2A, the onset of the positive inotropic effect (1 μmol/liter) required approximately 5 minutes; it was sustained during the period of drug exposure (16 minutes) and was reversible after washout. A single, maximally effective concentration of insulin (1 μmol/liter) increased the twitch tension to 154 ± 5% (range 110% to 207%) of the baseline value ($n = 28$, $p < 0.05$). There were no significant differences in inotropic responses to insulin between trabeculae from hearts of patients with dilated or ischemic cardiomyopathy (+52 ± 5% vs +62 ±

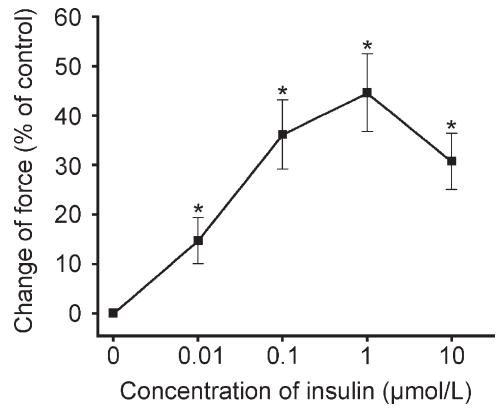

Figure 1. Cumulative concentration–response curves for insulin (0.01 to 10 μmol/liter, $n = 6$ preparations from 5 hearts). *$p < 0.05$ vs baseline before insulin administration. Time of exposure to each concentration of insulin was 10 minutes.

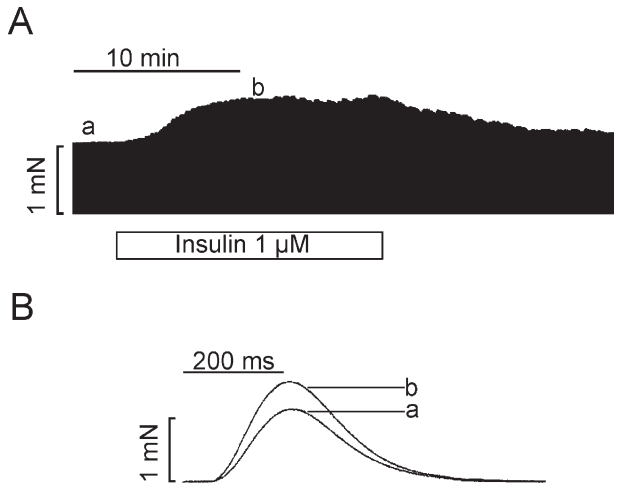

Figure 2. (A) Effect of insulin (1 μmol/liter) on isometric twitch tension in a muscle strip from an end-stage failing human heart (48-year-old man with dilated cardiomyopathy). (B) Traces of twitch curve at fast speed before (a) and after (b) insulin exposure are superimposed.

12%, $p > 0.05$). In addition, insulin exerted similar inotropic effects between the myocardia of diabetic and nondiabetic patients (+55 ± 8% vs +51 ± 5%, $p > 0.05$).

Although insulin increased the developed isometric tension and rates of contraction and relaxation, it did not significantly change the twitch kinetics, except for a slight shortening at the final phase of relaxation (Table 2 and Figure 2B). The action potential parameters (Table 3) were not changed significantly, except for a slight lengthening of the action potential duration at 90% repolarization (APD_{90}). After a long period (about 30 minutes) of washout, re-administration of insulin (1 μmol/liter) again resulted in a positive inotropic response (traces not shown), although this was lower than the initial response (+55 ± 8% vs +34 ± 7%; $p < 0.05$; $n = 9$). Thus, insulin had a tachyphylactic effect on the positive inotropic action in failing human myocardium. In four experiments, all glucose in the perfusate was removed. The positive inotropic effect of insulin (1 μmol/liter) was significantly lower in the glucose-free group than in controls (+85 ± 13% vs +55 ± 5%; $p < 0.05$; traces not shown).

Effect of Insulin on Catecholamine Release and β-Adrenergic Receptor Stimulation

To rule out any possibility of catecholamine release and subsequent stimulation of β-adrenergic receptors by insulin, we also tested insulin in the presence of propranolol, a non-selective β-adrenergic receptor blocker. Treatment with propranolol (1 μmol/liter) alone resulted in a significant decline in twitch force (by 29 ± 6%) and a slight shortening of relaxation times (RT_{90}; Table 4). As shown in Figure 3A and D, the positive inotropic effect of 1 μmol/liter insulin (+65 ± 14%; $p < 0.05$; $n = 6$) was not affected in the presence of propranolol.

Table 3. Influence of Insulin on Parameters of Action Potential

	APA (mV)	APD_{20} (ms)	APD_{50} (ms)	APD_{90} (ms)
Baseline	115 ± 7	155 ± 13	271 ± 21	404 ± 26
Insulin (1 μmol/liter)	118 ± 7	160 ± 17	288 ± 26	424 ± 29[a]

Values are mean ± SEM of 10 experiments. APD_{20}, APD_{50}, and APD_{90} are action potential durations at 20%, 50% and 90% repolarization, respectively. APA, amplitude of AP.

[a] $p < 0.05$, significantly different from the respective baseline value before insulin (1 μmol/liter) infusion by paired Student's *t*-tests.

Effects of Insulin on Intracellular Ca^{2+} Handling and Trans-sarcolemmal Ca^{2+} Influx

Although an increase in intracellular calcium transient ($[Ca^{2+}]_i$) was demonstrated as the underlying mechanism for the positive inotropic effect of insulin in the failing human myocardium,[11] the effects of mechanisms involved in the increase in $[Ca^{2+}]_i$ on short-term administration of insulin were unclear. We therefore assessed the contribution of trans-sarcolemmal Ca^{2+} influx to the inotropic responses of insulin. We first investigated L-type Ca^{2+} channels ($I_{Ca,L}$) by ruptured whole-cell voltage-clamp techniques in isolated human ventricular myocytes. Figure 4A shows typical current recordings during depolarization steps from –50 to +20 mV in a single myocyte. Insulin (1 μmol/liter) increased the amplitude of the inward Ca^{2+} current, whereas the decay kinetics remained unchanged. Similar experiments were performed in 7 ventricular myocytes from 5 hearts. Figure 4B shows current–voltage (I–V) relationships for the whole-cell $I_{Ca,L}$ measured before and after administration of insulin (1 μmol/liter) to the bath.

Control and insulin-treated current–density relationships showed bell-shaped curves and voltage dependence. The basal peak current density induced by voltage steps from −50 to +20 mV was 3.74 ± 0.44 pA/pF; insulin significantly increased the $I_{Ca,L}$ amplitude to 126 ± 6% (p

Table 2. Influence of Insulin on Basal Twitch Force and Kinetics

	F (%)	TPT (ms)	RT_{50} (ms)	RT_{90} (ms)	$+dF/dt_{max}$ (%)	$-dF/dt_{max}$ (%)
Baseline	100	263 ± 12	153 ± 5	317 ± 10	100	100
Insulin (1 μmol/liter)	150 ± 5[a]	266 ± 11	152 ± 5	307 ± 8[a]	143 ± 6[a]	149 ± 8[a]

F, force of contraction compared with baseline (%); TPT, time-to-peak tension (ms); RT_{50}, time to 50% relaxation from peak tension (ms); RT_{90}, time to 90% relaxation from peak tension (ms); $+dF/dt_{max}$, maximum rate of force increase; $-dF/dt_{max}$, maximum rate of force decline.

[a] $p < 0.05$ significantly different from the respective baseline value before insulin (1 μmol/liter) infusion by paired Student's *t* tests.

Table 4. Influence of Pharmacologic Blockers on Basal Twitch Force and Relaxation

Blockers	ΔF (%)	ΔTPT (%)	ΔRT_{50} (%)	ΔRT_{90} (%)
Propranolol (1 μmol/liter)	−29 ± 6[a]	0 ± 2	−6 ± 1[a]	−2 ± 1
Diltiazem (5 μmol/liter)	−35 ± 4[a]	−2 ± 4	12 ± 7	17 ± 8
Ryanodine (0.1 μmol/liter)	−62 ± 4[a]	15 ± 4[a]	−3 ± 5	14 ± 8
Cyclopiazonic acid (10 μmol/liter)	−32 ± 8[a]	2 ± 9	2 ± 7	20 ± 8[a]
HOE642 (10 μmol/liter)	−31 ± 4[a]	−4 ± 2	2 ± 3	6 ± 3
SEA0400 (1 μmol/liter)	−17 ± 5[a]	4 ± 5	5 ± 8	7 ± 7

ΔF indicates change in force of contraction; ΔTPT, change in time-to-peak tension; ΔRT_{50}, change in time to 50% relaxation from peak tension; ΔRT_{90}, change in time to 90% relaxation from peak tension.

[a] $p < 0.05$, significantly different from the respective baseline value before drug infusions by paired Student's *t*-tests.

< 0.05) of the basal value. Fast (τ_1) and slow (τ_2) inactivation time (t) constants were not different in either the absence or presence of insulin. At +20 mV, time constants were 10.6 ± 2.7 milliseconds (τ_1) and 36.6 ± 6.8 milliseconds (τ_2) in control, and 11.0 ± 2.7 milliseconds (τ_1) and 40.4 ± 7.8 milliseconds (τ_2) after addition of insulin to the extracellular solution ($p > 0.05$, $n = 7$).

To determine whether the positive inotropic action of insulin was dependent on calcium influx via L-type Ca^{2+} channels, its effect was studied in isolated myocardium perfused with medium containing diltiazem (5 μmol/liter), a well-known L-type Ca^{2+} channel blocker.[23] At this concentration, diltiazem exerted a pronounced negative inotropic effect (Table 4). Applied at steady-state contractile conditions, the positive inotropic response of insulin (1 μmol/liter) was not affected by the presence of diltiazem (+65 ± 11%; $p < 0.05$; $n = 8$; Figure 3B and D).

Intracellular Ca^{2+} accumulation can also be modulated by Na^+-Ca^{2+} exchange. This electrogenic ion transporter extrudes Ca^{2+} for Na^+ influx in its forward mode, but may also work in its reverse mode, resulting in Ca^{2+} influx during the depolarization and contraction of failing human ventricular myocytes.[19] The latter might be favored by an increase in $[Na^+]_i$. In this setting, pre-incubation of muscle strips with the Na^+-H^+ exchange inhibitor HOE642[24] (10 μmol/liter) significantly affected basal force (Table 4) and resulted in a significant reduction in the maximal inotropic response to insulin by 37 ± 11% ($p < 0.05$; Figure 5B and D). In a further set of experiments, the effects of a selective inhibitor of the Na^+-Ca^{2+} exchanger SEA0400[25] were tested, as shown in Figure 5C. SEA0400 (1 μmol/liter) depressed the basal force of contraction significantly (Table 4) and the positive inotropic effect of insulin was reduced by 41 ± 8% ($p < 0.05$; Figure 5C and D).

Effects of Insulin on Sarcoplasmic Reticulum Ca^{2+} Handling

Participation of the sarcoplamic reticulum (SR) in events leading to the development of the positive inotropic action of insulin was tested by studying the effect of insulin (1 μmol/liter) on myocardium in the presence of 0.1 μmol/liter ryanodine (to inhibit Ca^{2+} released from the SR) or 10 μmol/liter cyclopiazonic acid (CPA; to inhibit Ca^{2+} re-uptake by SR Ca^{2+}-ATPase). Ryanodine resulted in a significant decline in twitch force (by 62%) and prolongation of time-to-peak tension (Table 4). As shown in Figure 3C and D, the positive inotropic effect of insulin (56 ± 14%; $p < 0.05$; $n = 8$) was not affected by ryanodine. However, it was partially inhibited (34 ± 8%; $p < 0.05$; $n = 5$) by cyclopiazonic acid (Figure 5A and D).

DISCUSSION

The main findings from this study are that insulin-mediated positive inotropy was not related to catecholamine release and subsequent stimulation of β-adrenergic receptors, but it was partially independent of glucose. Furthermore, it could be related to the enhanced activity of SR Ca^{2+}-ATPase, trans-sarcolemmal Ca^{2+} entry mainly via reverse-mode Na^+-Ca^{2+} exchange, and an insulin-mediated activation of Na^+-H^+ exchange. Because we had no normal human heart tissues for comparison, the aforementioned results are only applicable to the failing human heart and may not necessarily apply to normal cardiomyocytes.

Insulin exerts acute hemodynamic as well as long-term genomic effects on the cardiovascular system. Insulin infusion has been used in the past to treat severe heart failure.[26] Parsonage et al[3] demonstrated a beneficial hemodynamic effect of insulin in patients with chronic heart failure, but it had a negligible effect on sympathetic nerve system activity when measured in terms of plasma adrenaline concentration. The fall in heart rate suggests an increase in stroke volume, which may represent an improvement in contractility mediated by insulin.[27]

We observed a ~50% increase in isometric twitch force at 1 μmol/liter insulin with little change in twitch kinetic parameters except for a slight shortening during the final phase of relaxation (RT_{90}). The acute functional effects of insulin are substantial and of potential clinical relevance. The magnitude of the inotropic responses to insulin in this study is similar to effects reported previously for the isolated myocardium.[5,6,10] The positive inotropy of insulin started at a concentration of 0.01 μmol/liter and was maximal at 1 μmol/liter. These concentrations of insulin

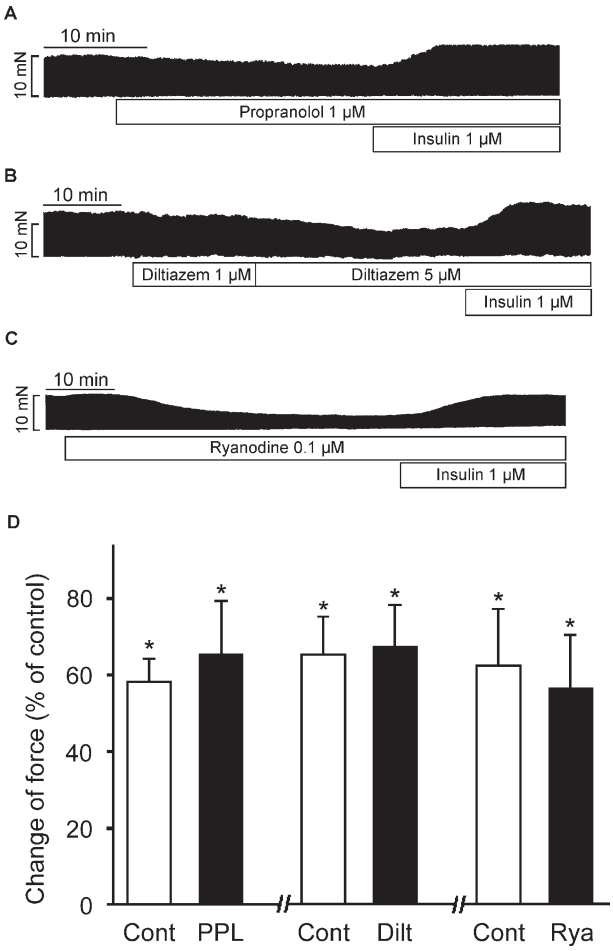

Figure 3. Effects of insulin (1 μmol/liter) on contractile force of ventricular tissues pre-incubated with: (A) 1 μmol/liter propranolol; (B) 5 μmol/liter diltiazem; and (C) 0.1 μmol/liter ryanodine. Insulin reversed the depressant effect of these drugs. (D) Effect of insulin (1 μmol/liter) on contractile force after pre-incubation with propranolol (PPL, 1 μmol/liter; filled bar), diltiazem (Dil, 5 μmol/liter; filled bar) and ryanodine (Rya, 0.1 μmol/liter; filled bar). Experiments were performed in 6 preparations from 5 hearts, 8 preparations from 6 hearts and 8 preparations from 4 hearts, respectively. The effect of insulin on the contractile force of control (Cont, open bars) muscles without pre-incubation (from the same heart) is shown for comparison. *$p < 0.05$ vs baseline before insulin administration.

are identical to those used in experiments on isolated rat ventricular myocytes,[16] and similar to those applied to isolated canine[5] and piglet[10] myocardium. These pharmacologic doses effective in vitro are higher than the insulin concentrations normally found in blood, which range from 0.3 to 3 nmol/liter.[28] We cannot explain this difference in insulin doses between in vivo and in vitro investigations. However, cardiac tissue has been reported to contain a 100-fold higher insulin concentration than blood, indicating that the concentrations of insulin used in this and other studies are physiologically relevant. Some commercial insulin preparations contain phenol or *m*-cresol (3 methyl-phenol) as a preservative, which exerts a negative inotropic effect, and this may affect the experimental results.[9,10,29,30]

Similar to previous studies,[5,6,8,10] we have shown that the positive inotropic effect of insulin was partially preserved in the absence of glucose, suggesting that its effects in the failing human myocardium are not entirely related to its ability to facilitate transport of glucose across the myocardial cell and that the hormone may affect other mechanisms that alter myocardial contractility. The results of the present study do not rule out an important role of insulin upon glucose transport, but suggest that a second mechanism, independent from metabolic factors, might play a significant role in the hormone's inotropic action.

Because propranolol, a non-specific β-adrenergic-receptor blocker, failed to modify the positive inotropic effect of insulin, the release of catecholamine and subsequent activation of β-adrenergic receptors[5,7,31,32] seems unlikely to be a mode of action of insulin in the heart. Although our study was performed in vitro, similar results were reported by Lucchesi et al[5] and

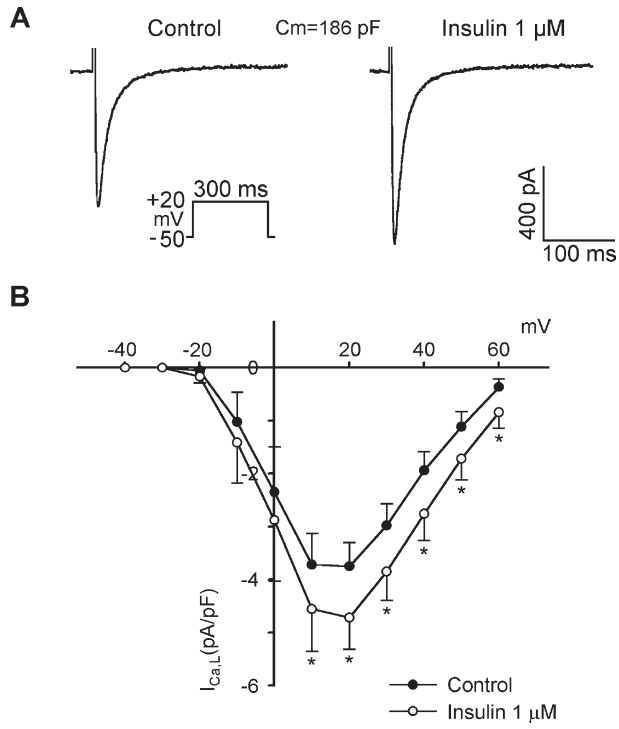

Figure 4. Effects of insulin (1 μmol/liter) on L-type calcium current ($I_{Ca,L}$) of human ventricular myocytes. (A) Original recording of $I_{Ca,L}$ measured at +20 mV in the absence (left) and presence (right) of insulin. The holding potential was –50 mV. The cell membrane capacitance of the cardiomyocyte was 186 pF. (B) Current–voltage (I–V) relationships obtained for peak calcium current in control conditions and in the presence of insulin. The I–V curve was obtained by plotting peak current densities (pA/pF) against test pulse potential. Experiments were performed in 7 myocytes from 5 failing hearts. Symbols and bars represent mean ± SEM. *$p < 0.05$ vs baseline before insulin administration.

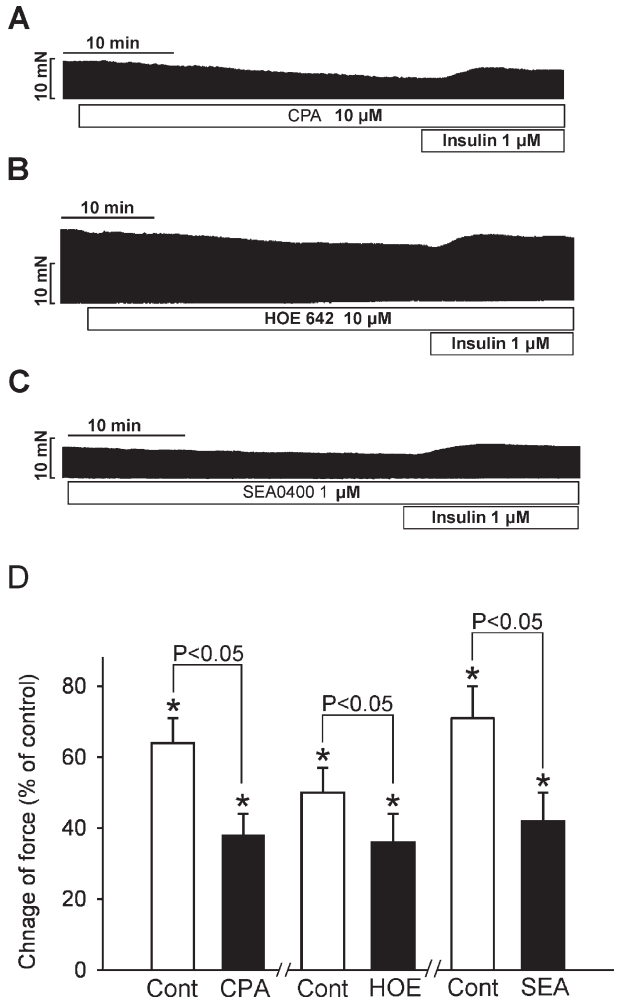

Figure 5. Effects of insulin (1 μmol/liter) on the contractile force of ventricular tissues pre-incubated with: (A) cyclopiazonic acid (CPA, 10 μmol/liter); (B) HOE642 (HOE, 10 μmol/liter); and (C) SEA0400 (SEA, 1 μmol/liter). Insulin failed to reverse the depressant effect of these drugs. (D) Effects of insulin on contractile force after pre-incubation with cyclopiazonic acid (CPA, 10 μmol/liter; filled bar), HOE642 (HOE, 10 μmol/liter; filled bar) and SEA0400 (SEA, 1 μmol/liter; filled bar). Experiments were performed in 7 preparations from 4 hearts, 9 preparations from 4 hearts and 8 preparations from 6 hearts, respectively. Effects of insulin on contractile force of control (Cont, open bars) muscles without pre-incubation (from the same heart) are shown for comparison. *$p <$ 0.05 vs baseline before insulin administration.

Kerns et al[31] in a canine model (in vivo) of acute β-blocker toxicity. In addition, Kerns et al[31] found that insulin is a better antidote in such situations than glucagon or epinephrine. Our results are consistent with previous studies in animal models[5,7,31,32] showing that insulin is able to reverse the negative inotropic effect of propranolol. In addition, insulin might modulate the inotropic response of β-adrenergic receptor agonists in mammalian hearts. A low concentration of insulin (0.3 nmol/liter) sensitized the positive inotropy of isoproterenol in the isolated rat papillary muscle,[33] but a high concentration (6 μmol/liter) impaired the inotropic effect of norepinephrine in cat papillary muscle.[10] Baltensperger et al[34] showed that insulin is able to stimulate insulin receptor–catalyzed phosphorylation of the β_2-adrenergic receptor. Aulbach et al[16] reported that cAMP and cAMP-dependent protein kinase (PKA) are involved in the stimulation of $I_{Ca,L}$ by insulin in rat ventricular myocytes. Thus, it is possible that there are interactions between insulin and the β-adrenergic receptor at the post-receptor level via G proteins,[34] phosphodiesterase and cAMP.[16]

In view of the importance of Ca^{2+} in muscle contraction and relaxation, these results indicate that insulin might increase both the rate and amount of Ca^{2+} availability as well as removal from the cytoplasm in the myocardial cell. Previous studies[7,11] suggested that the positive inotropic action of insulin may be mediated via the process of translocation of Ca^{2+} from the binding sites to contractile proteins and thus increase the amount of Ca^{2+} available to the myofilaments for augmenting the force of contraction. This concept is consistent with the results of the present study showing that, after addition of insulin to the muscle bath, both isometrically developed tension and the maximal rate of development of tension increased, but there was no significant change in the time-to-peak tension. This mimics the effects of increasing extracellular Ca^{2+} concentration. Thus, it is evident that the extracellular source of Ca^{2+} may play a central role in the development of the positive inotropic action of insulin.[7]

Recently, Aulbach et al[16] demonstrated a substantial increase in L-type Ca^{2+} channel activity in rat ventricular myocytes upon exposure to insulin at room temperature. In our studies, L-type Ca^{2+} currents also increased significantly in isolated human ventricular myocytes upon exposure to insulin at a physiologic temperature (35 ± 1°C); however, this could not be prevented by diltiazem. Recent studies have shown that a glucose–insulin–potassium (GIK) solution has more beneficial effects than other inotropic agents in treating patients with calcium channel blocker intoxication (see later).[32] Insulin might affect other mechanisms in addition to the voltage-sensitive L-type Ca^{2+} channels for increasing Ca^{2+} influx into the cell (reverse-mode Na^+-Ca^{2+} exchange or excitation–contraction coupling downstream of $I_{Ca,L}$).[35]

Although Na^+-Ca^{2+} exchange is thought to be involved mainly in the efflux of Ca^{2+} from myocytes in the failing human myocardium, there is evidence for its role in both Ca^{2+} influx and efflux.[19,36] It is conceivable that insulin may affect sarcolemmal Na^+-Ca^{2+} exchange,[7,11,13] and thus promote a rise and fall in the concentration of Ca^{2+} in the cytoplasm to increase the rates of contraction and relaxation, respectively. Inotropic responses to insulin could be reduced to a signifi-

cant extent by inhibition of Na^+-H^+ exchange with HOE642. A previous study with insulin-like growth factor-1 (IGF-1) reported that activation of Na^+-H^+ exchange may contribute to the positive inotropic effects of insulin on the human myocardium, possibly via enhanced Ca^{2+} entry through $[Na^+]_i$-dependent activation of reverse-mode Na^+-Ca^{2+} exchange.[37] This was supported in a recent study on rat ventricular myocytes, in which insulin increased pH_i through activation of Na^+-H^+ exchange.[38] Similar to the results of von Lewinski et al,[11] our study has demonstrated that another selective inhibitor of the Na^+-Ca^{2+} exchange, SEA0400 (1 μmol/liter), partially prevented the inotropic response of the failing human myocardium to insulin. Thus, it appears that Na^+-Ca^{2+} exchange may be intimately involved in the positive inotropic action of insulin. The possibility that insulin receptors in the sarcolemmal membrane might be coupled with the Na^+-Ca^{2+} exchange system remains to be investigated.

In this study we found that a low concentration of ryanodine (0.1 μmol/liter) did not prevent the increase in contractile force development by insulin. These findings exclude the involvement of SR Ca^{2+}-release channels (ryanodine receptors) in the action of insulin on the failing human heart. It should be noted that insulin stimulates the Ca^{2+}-ATPase pump of the SR,[14,15] and this effect could explain the increase in the rate of relaxation (RT_{90}) of the myocardium by insulin (albeit small; see Figure 2B and Table 2). We also observed that the positive inotropic effect of insulin was partially inhibited under depression with cyclopiazonic acid (CPA). These observations are consistent with the view that the effects of insulin on the myocardium are intimately associated with enhancing SR Ca^{2+} uptake in the cell.

Insulin has been reported to increase muscle contractile force at low calcium concentrations (1.25 mmol/liter) while decreasing it at high calcium concentrations (5 mmol/liter).[6] In this regard, cardiac preparations show decreased contractile force with intracellular Ca^{2+} overload.[39] Thus, the effects of insulin on the myocardium are intimately associated with changes in Ca^{2+} movement in the cell. However, sensitization of the myofilaments does not appear to be a major mode of action of insulin in the failing human myocardium, because the relaxation time in the present study barely changed in the presence of insulin (Table 2).

There is increasing evidence to support the beneficial effects of GIK solution in treating patients with acute myocardial infarction,[40] cardiac surgery[41] and septic shock,[42] although one recent large-scale clinical trial did not support this finding (2005 CREATE-ECLA Trial).[43] GIK solution showed a beneficial effect in an animal model of β-blocker intoxication[31] and among patients with calcium channel blocker intoxication,[32] but the underlying mechanism remains unclear. The present results demonstrate that insulin is able to reverse the contractile force of depressed human myocardium in the presence of propranolol or diltiazem. These findings may offer an explanation for the beneficial hemodynamic effects of GIK solution in the treatment of cardiovascular disorders.

Limitations

One limitation of the present study is that there was tachyphylaxis of the cardiac contractile response to insulin,[8] so we should analyze the experimental results with caution. A further limitation of our study is that we did not measure aequorin light signals for comparison of changes in $[Ca^{2+}]_i$ to development of twitch force. Although normal human hearts were unavailable for the present study, we also noted insulin-mediated positive inotropy in our recent study on normal canine papillary muscles.[30]

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