Insulin Causes \([Ca^{2+}]_i\)-Dependent and \([Ca^{2+}]_i\)-Independent Positive Inotropic Effects in Failing Human Myocardium

Dirk von Lewinski, MD; Sebastian Bruns; Stefanie Walther; Harald Kögler, MD; Burkert Pieske, MD

Background—Insulin has been shown to exert positive inotropic effects in several in vitro and in vivo models, but signal transduction and substrate dependency remain unclear. We examined inotropic responses and signal transduction mechanisms of insulin in human myocardium.

Methods and Results—Experiments were performed in isolated trabeculae from end-stage failing hearts of 58 nondiabetic and 3 diabetic patients undergoing heart transplantation. The effect of insulin (0.3 and 3 IU/L) on isometric twitch force (37°C, 1 Hz) was tested in the presence of glucose or pyruvate as energetic substrate. Furthermore, intracellular \(Ca^{2+}\) transients (aequorin method), sarcoplasmic reticulum (SR) \(Ca^{2+}\) content (rapid cooling contractures), and myofilament \(Ca^{2+}\) sensitivity (semiskinned fibers) were assessed. In addition, potential signaling pathways were tested by blocking glycolysis, PI-3-kinase, protein kinase C, diacylglycerol kinase, insulin-like growth factor-1 receptors, or transsarcolemmal \(Ca^{2+}\) entry via the \(Na^{+}/Ca^{2+}\) exchanger. Insulin exerted concentration-dependent and partially substrate-dependent positive inotropic effects. The phosphatidylinositol-3-kinase inhibitor wortmannin and the \(Na^{+}/Ca^{2+}\) exchanger reverse-mode inhibitor KB-R7943 completely or partially prevented the functional effects of insulin. In contrast, insulin-like growth factor-1 receptor blockade, protein kinase C inhibition, and diacylglycerol kinase blockade were without effect. The inotropic response was associated with increases in intracellular \(Ca^{2+}\) transients, SR \(Ca^{2+}\) content, and increased myofilament \(Ca^{2+}\) sensitivity.

Conclusions—Insulin exerts \(Ca^{2+}\)-dependent and \(Ca^{2+}\)-independent positive inotropic effects through a phosphatidylinositol-3-kinase-dependent pathway in failing human myocardium. The increased \([Ca^{2+}]_i\) originates at least in part from enhanced reverse-mode \(Na^{+}/Ca^{2+}\) exchange and consequently increased SR-\(Ca^{2+}\) load. These nongenomic functional effects of insulin may be of clinical relevance, eg, during insulin-glucose-potassium infusions. (Circulation. 2005;111:2588-2595.)

Key Words: insulin ■ calcium ■ heart failure ■ myocardium ■ contractility

Patients with chronic heart disease often have insulin resistance, diabetes, or altered glucose metabolism.\(^1\) Insulin, a polypeptide of 51 amino acids, is known to regulate serum glucose levels, protein synthesis, and growth. Effects of insulin on myocardial function have been tested in several animal models under in vitro and in vivo conditions, and controversial results with both enhanced contractility and myocardial performance\(^2\)-\(^6\) or no functional effects\(^7\)-\(^8\) have been reported. Insulin infusions improved ventricular function in one study in humans\(^9\) but had no effect in another.\(^10\) In addition, the functional response to insulin may be different in diabetic versus nondiabetic animals\(^3\)-\(^12\) and humans.\(^9\),\(^13\)

Recently, the onset of insulin resistance has been shown to coincide with progression from pressure-overload hypertrophy to dilatation.\(^14\) Whole-body insulin resistance is prevalent in congestive heart failure patients with either ischemic heart failure or idiopathic dilated cardiomyopathy.\(^15\),\(^16\) Therefore, insulin resistance may contribute to contractile dysfunction by genomic, metabolic, and direct functional effects. Despite the controversial reports on direct effects of insulin on myocardial function in mammalian myocardium, no data are available that directly test the inotropic response to insulin in human myocardium. In addition, the mechanisms of action of the functional effect of insulin in mammalian myocardium remains controversial.\(^5\) Under in vivo conditions, improved global ventricular function during insulin infusions\(^9\) might exclusively result from insulin-dependent arteriolar vasodilatation with peripheral unloading\(^13\),\(^17\) and improved myocardial blood flow.\(^17\) Therefore, we directly assessed functional effects and mechanisms of action of insulin in isolated failing human myocardium. Our main findings were that insulin exerts both \(Ca^{2+}\)-dependent and \(Ca^{2+}\)-independent positive inotropic effects. These functional effects were related to activation of phosphatidylinositol-3-kinase (PI-3-kinase) and, in part, reverse-mode \(Na^{+}/Ca^{2+}\) exchange. These data help to improve our understanding of nongenomic hormonal effects in the human heart.
Muscle Strip Preparation
Small endocardial trabeculae were dissected from the left or right ventricle as described previously,\textsuperscript{18} connected to an isometric force transducer, and superfused with bicarbonate-containing Tyrode’s solution. Muscles were electrically stimulated at 1 Hz (37°C), and isometric contractions were recorded at optimum preload ($L_{\text{max}}$). The functional effects of insulin were assessed with 2 different concentrations (0.3 and 3 IU/L) and 2 different substrate conditions of the Tyrode solution (either 11.2 mmol/L glucose or 22.4 mmol/L pyruvate).

Aequorin Measurements
At steady state contractile function, the Ca$^{2+}$-regulated bioluminescent photoprotein aequorin was microinjected into the quiescent muscle as described previously.\textsuperscript{18} Aequorin light emission was detected with a photomultiplier, which was vertically mounted with its cathode just above the glass cuvette that contained the muscle. Aequorin light emission was detected with a photomultiplier, which was vertically mounted with its cathode just above the glass cuvette that contained the muscle.

Rapid Cooling Contractures
Rapid cooling contractures (RCCs) were elicited by a rapid decrease in the temperature of the muscle chamber from 37°C to 1°C by switching from a warm to a cold solution with solenoid pinch valves at the bath inlet as previously described.\textsuperscript{19} The resulting cooling contracture is an index for sarcoplasmic reticulum (SR) Ca$^{2+}$ content.

Semiskinned Fiber Preparations
Fibers were skinned by the technique and solutions described by Hambarchian et al.\textsuperscript{20} Saponin (50 µg/mL) was added for 45 minutes to functionally skin the trabeculae. This technique allows the control of intracellular ion concentrations but maintains sarcolemmal receptors and leaves subcellular signaling mechanisms intact.\textsuperscript{21} Concentration-response curves for Ca$^{2+}$ (tension-Ca$^{2+}$ relationship) were obtained in each muscle, first without insulin and after complete reequilibration in the presence of 3 IU/L insulin.

Drugs
Insulin (Insuman Rapid 100 IU/mL, Hoechst Marion Russel) was used as supplied. Insulin-like growth factor-1 (IGF-1) receptor antibody ($\alpha$-IR-3 clone; Oncogene Research Products) was dissolved as recommended by the manufacturer. KB-R 7943 (5 µmol/L; Tocris), diacylglycerol (DAG)-kinase inhibitor (1 µmol/L), chelerythrine (10 µmol/L), GF109203 (1 µmol/L), cyclopiazonic acid (10mmol/L), ryanodine (1 µmol/L), wortmannin (0.1 µmol/L), and iodoacetate (0.5 µmol/L; all from Sigma) were added to the organ bath 30 minutes before the experiment.

Statistical Analysis
Data are expressed as mean±SEM. Differences were compared by paired Student $t$ test or 1-way repeated-measures ANOVA followed by Student-Newman-Keuls test when appropriate. Statistical significance was taken as $P<0.05$.

Results
Inotropic Effects of Insulin
Insulin exerted pronounced transient positive inotropic effects in isolated human cardiac muscle (Figure 1A) without changes in diastolic tension. These functional effects of insulin were concentration dependent in glucose-containing solution, with a maximum increase in developed force of 10.6±1.8% at low insulin concentrations (0.3 IU/L; $n=8$) and 28.1±5.7% (of basal twitch force, respectively) at high insulin concentrations (3 IU/L; $n=8$; Figure 1B). In pyruvate-containing solution, the relative increase in twitch force with insulin was smaller but independent of the insulin concentration used (10.5±3.2%, $n=8$ and 13±3.8%, $n=9$, respectively; Figure 1B). Developed force at baseline conditions was significantly higher in the pyruvate group (50.3±10.6
Influence of Insulin on Twitch Kinetics

<table>
<thead>
<tr>
<th></th>
<th>TPT</th>
<th>RT 90</th>
<th>RT 30</th>
<th>+dT/dtmax</th>
<th>-dT/dtmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline glucose</td>
<td>233±18</td>
<td>137±9</td>
<td>345±31</td>
<td>100±27</td>
<td>90±32</td>
</tr>
<tr>
<td>Insulin 3 IU/L</td>
<td>229±15</td>
<td>140±9</td>
<td>363±32</td>
<td>142±49</td>
<td>113±37</td>
</tr>
<tr>
<td>Baseline pyruvate</td>
<td>286±11</td>
<td>170±7</td>
<td>603±23</td>
<td>253±75</td>
<td>204±59</td>
</tr>
<tr>
<td>Insulin 3 IU/L</td>
<td>279±11</td>
<td>182±8</td>
<td>581±23</td>
<td>279±80</td>
<td>214±59</td>
</tr>
</tbody>
</table>

TPT indicates time to peak tension (ms); RT 90, 50% relaxation from peak tension (ms); RT 30, 90% relaxation from peak tension (ms); +dT/dtmax, maximum rate of tension increase (mN/s/mm²); and -dT/dtmax, maximum rate of tension decline (mN · s⁻¹ · mm⁻²).

versus 25.7±6.5 mN/mm² in the presence of glucose). Insulin did not significantly affect twitch kinetics (Table) but tended to prolong relaxation times.

To further assess the underlying mechanisms of the substrate dependence, additional experiments were performed after glycolysis was blocked with iodoacetate (0.5 μmol/L). In muscles preincubated with higher concentrations of iodoacetate (1 μmol/L), muscles developed contracture within the first minutes. The insulin-mediated positive inotropic effect was reduced by about 50% in the glucose group in the presence of 0.5 μmol/L iodoacetate and was comparable to the insulin-mediated effect in the pyruvate group under this experimental condition. These data show that at least some of the insulin-dependent positive inotropic effects are not related to increased glycolysis.

Additional experiments were performed in 9 trabeculae from 3 hearts obtained from patients with diabetes mellitus. Insulin exerted identical inotropic effects in both glucose- and pyruvate-containing solutions compared with nondiabetic myocardium (at 3 IU/L, 33±13% [n=6] and 15.8±11% [n=3], respectively).

Ca²⁺-Dependent and Ca²⁺-Independent Effects of Insulin

All further experiments designed to test the subcellular mechanisms of action of insulin were performed in glucose-containing Tyrode’s solution at an insulin concentration of 3 IU/L. First, experiments were performed in aequorin-loaded muscle strips. Figure 2A shows original tracings of the effects of insulin on twitch force and aequorin light signals. The inotropic effect of insulin (23.2±10%; P<0.05) was associated with a parallel, albeit smaller increase in aequorin light emission (15.8±4.2% of the basal value, P<0.05; Figure 2B). These data demonstrate that the functional effects of insulin are related to increased [Ca²⁺], at least in part.

We then compared these data to results from an intervention that increases force by an exclusively Ca²⁺-dependent mechanism, ie, elevated [Ca²⁺]o. Increasing [Ca²⁺]o from 2.5 to 3.2 mmol/L (Figure 2B) resulted in an increase in twitch force by 23.7±4.3% (n=8; P<0.05) and an increase in aequorin light emission by 20.5±7.2% (P<0.05). Although not significantly different between groups, for an almost identical increase in force, the increase in aequorin light was smaller with insulin than with [Ca²⁺]o (3.2 mmol/L). This was quantified by calculating the relative increase in force (F) versus aequorin light emission (L). ΔF/ΔL was 0.98±0.05 with elevated [Ca²⁺]o, and 1.34±0.18 with insulin (P<0.05). These findings, in association with the tendency to prolonged relaxation kinetics, may point to an additional Ca²⁺-sensitizing effect of insulin. Interestingly, we have previously shown that IGF-1 does not affect Ca²⁺ sensitivity compared with 4 mmol/L [Ca²⁺], (ΔF/ΔL was 1.02±0.38 and 1.03±0.12, respectively), but β-adrenergic stimulation reduced the relative increase in ΔF/ΔL to 0.56±0.11.

To further clarify the potential role of increased myofilament Ca²⁺ sensitivity after insulin administration, we directly assessed Ca²⁺ sensitivity in semiskinned fibers (n=5 from failing human hearts). Insulin significantly shifted the tension-[Ca²⁺]o relationship to the left, which resulted in a decreased EC₅₀ (4.75±0.53 μmol/L) in the presence of insulin versus control (6.19±0.79 μmol/L, P<0.05; Figure 3).

Subcellular Mechanisms of Action of Insulin

We performed rapid-cooling experiments to directly assess the effects of insulin on SR Ca²⁺ content. Figure 4A shows a representative original recording. The upper tracing reflects temperature near the surface of the muscle, the lower tracing twitch force. At steady state isometric contractions, the muscle was cooled to ~1°C, and a stable cooling contracture as an index for SR Ca²⁺ content developed. In these experiments, we used paired cooling contractures to additionally test whether insulin directly affected SR Ca²⁺ uptake. The rationale for these experiments is that during the rewarming period (in the unstimulated muscle), cytosolic Ca²⁺ is competively removed from the cytosol by sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA2a) and Na⁺/Ca²⁺-ATPase.

Figure 2. A, Superimposed original tracings of effect of insulin (3 IU/L) on isometric twitch tension (left) and intracellular Ca²⁺ transients (aequorin light signals, right). B, Effects of insulin (3 IU/L, left) and increased [Ca²⁺]o, (right) on twitch force (solid bars) and aequorin light emission (open bars). Average data from 6 preparations from 3 hearts. *P<0.05 vs baseline.
exchange. On rewarmin, the muscle completely relaxed, and the experimental protocol was repeated after addi-

tion of insulin. The average data are presented in Figure 4B. Insulin (3 IU/L) significantly increased twitch force and RCCs in glucose-containing solution by 42±7.1% and 15.7±4.9%, respectively. Additional experiments were performed in 6 prepara-

tions from 6 hearts after SR function was blocked with cyclopiazonic acid and ryanodine (Figure 4B). This intervention completely prevented RCCs and reduced the insulin-dependent positive inotropic effect by ≈46%. This indicates that the inotropic effect of insulin is associated with but does not exclusively depend on increases in SR Ca2+ content.

The RCC2/RCC1 ratio in failing human trabeculae was 47% before and 47% in the presence of insulin 3 IU/L. This indicates that ≈47% of the cytosolic Ca2+ is reaccumulated into the SR, whereas ≈53% is eliminated from the cytosol via Na+/Ca2+ exchange, and that this ratio is not affected by insulin. These numbers are typical for failing human myocardium.19

To further elucidate the insulin-dependent signal transduc-

tion pathways, the functional effect of insulin was compared with the effect of 0.1 μmol/L IGF-122 without and with blockade of either the IGF-1 receptors with IGF-1 receptor antibody (α-IR-3) or PI-3-kinase with wortmannin (0.1 μmol/L). Preincubation with the antibody only blunted IGF-1–mediated inotropy, whereas wortmannin almost completely prevented the increase in twitch force after both insulin and IGF-1 (Figure 5).

Insulin-mediated positive inotropic effects were not af-

forced by DAG-kinase inhibition (n=6) and were blocked neither by the unspecific protein kinase C (PKC) inhibitor chelerythrine (n=4) nor GF109203, which more specifically blocks typical (α,β1) and new (δ, ε) isofoms of PKC (the

inotropic response to insulin was 26.8±6.4% in the presence of GF109203X and 22.2±5.4% for control experiments in muscles from the same hearts; n=9 for each group; P=NS; Figure 6).

Intracellular Ca2+ handling may be modulated by the Na+/Ca2+ exchanger. This electrogenic ion transporter ex-

trudes Ca2+ for Na+ influx in its forward mode but may also work in its reverse mode, resulting in Ca2+ influx during depolarization.23 Preincubation with the reverse-mode Na+/ Ca2+ exchange inhibitor KB-R 7943 (5 μmol/L; n=8) re-

duced the positive inotropic effect of insulin by 62.1±6.3% (P<0.05; Figure 6). These data indicate that part of the inotropic response to insulin is related to reverse-mode Na+/Ca2+ exchange activation.

Discussion

This is the first report on functional effects of insulin in isolated human myocardium. The results show that (1) insulin exerts a concentration-dependent positive inotropic effect that contains a Ca2+-dependent and a Ca2+-independent component; (2) the inotropic effect is partially substrate dependent; and (3) the positive inotropic effect involves PI-3-kinase and reverse-mode Na+/Ca2+ exchange activation but is independent from DAG or PKC activation.
whether improved ventricular performance in the human peripheral and coronary circulation remains unknown. Insulin also exerts direct vasodilatory effects in the cardiovascular system. Genomic effects include induction of endothelial dysfunction and antiapoptotic signaling. Acute application of insulin to human healthy volunteers or patients with diabetes increases left ventricular diastolic tension. The magnitude of the inotropic responses to insulin in the present study is comparable to the effects reported in isolated guinea pig and rat hearts, even though the lower concentration used is up to 5 times higher than postprandial plasma insulin levels in healthy volunteers. However, local insulin concentrations at the receptor level are unknown and may be higher than plasma levels. In addition, higher plasma insulin concentrations may be reached under clinical conditions such as intravenous insulin application (eg, insulin-glucose-potassium infusions).

We observed a concentration-dependent positive inotropic effect of insulin in isolated end-stage failing human myocardium that accounted to up to 25% increase in twitch force at 3 IU/L insulin. The inotropic effect was associated with minor prolongations of relaxation time and no change in diastolic tension. The magnitude of the inotropic responses to insulin in the present study is comparable to the effects reported in isolated guinea pig and rat hearts.

The acute functional effects of insulin in the present study were substantial and may be of functional relevance: maximal inotropic effects in the same system. In addition, the inotropic response to insulin was identical in end-stage failing myocardium from patients with long-standing diabetes mellitus.

However, we also observed a partial substrate dependency of the inotropic effect. The maximal inotropic response to insulin was 50% smaller in pyruvate-containing Tyrode’s solution than in glucose-containing Tyrode’s solution at the higher insulin concentration. In addition, blocking glycolysis in muscles preincubated with glucose attenuated the inotropic response to insulin to the extent seen in the presence of pyruvate. We therefore suggest that the functional response to insulin may be related to 2 components: one that originates from improved glucose utilization and metabolism, and one that is independent from metabolic factors. However, we are not aware to what extent we have reduced glucose metabolism at the iodoacetate concentration used in these experiments, because higher concentrations of the inhibitor induced irreversible contracture of the muscles. Furthermore, basal developed force was significantly higher in the pyruvate group. This is in line with the described positive inotropic effect of pyruvate. With the higher basal twitch tension, an additional positive inotropic response may appear smaller if calculated as a percentage of basal force. In fact, the difference in the average increase in twitch force with 3 IU/L insulin was also less pronounced if absolute values were calculated: force increased by 4.3 mN/mm² in glucose-containing solution and by 3.3 mN/mm² in pyruvate-containing solution.

Signal Transduction Pathways of Insulin in Human Myocardium
In addition to binding to sarcolemmal insulin receptors, insulin is able to bind to both types of IGF receptors.

We have previously shown that IGF-1 binding to the IGF-1

β-adrenoceptor stimulation in isolated failing human myocardium increases force by 100%, and therefore, the inotropic effect observed in this study with insulin accounts for up to one fourth of the maximal response to catecholamines in the same system. In addition, the inotropic response to insulin was identical in end-stage failing myocardium from patients with long-standing diabetes mellitus.

However, we also observed a partial substrate dependency of the inotropic effect. The maximal inotropic response to insulin was 50% smaller in pyruvate-containing Tyrode’s solution than in glucose-containing Tyrode’s solution at the higher insulin concentration. In addition, blocking glycolysis in muscles preincubated with glucose attenuated the inotropic response to insulin to the extent seen in the presence of pyruvate. We therefore suggest that the functional response to insulin may be related to 2 components: one that originates from improved glucose utilization and metabolism, and one that is independent from metabolic factors. However, we are not aware to what extent we have reduced glucose metabolism at the iodoacetate concentration used in these experiments, because higher concentrations of the inhibitor induced irreversible contracture of the muscles. Furthermore, basal developed force was significantly higher in the pyruvate group. This is in line with the described positive inotropic effect of pyruvate. With the higher basal twitch tension, an additional positive inotropic response may appear smaller if calculated as a percentage of basal force. In fact, the difference in the average increase in twitch force with 3 IU/L insulin was also less pronounced if absolute values were calculated: force increased by 4.3 mN/mm² in glucose-containing solution and by 3.3 mN/mm² in pyruvate-containing solution.

Signal Transduction Pathways of Insulin in Human Myocardium
In addition to binding to sarcolemmal insulin receptors, insulin is able to bind to both types of IGF receptors.

We have previously shown that IGF-1 binding to the IGF-1

β-adrenoceptor stimulation in isolated failing human myocardium increases force by 100%, and therefore, the inotropic effect observed in this study with insulin accounts for up to one fourth of the maximal response to catecholamines in the same system. In addition, the inotropic response to insulin was identical in end-stage failing myocardium from patients with long-standing diabetes mellitus.

However, we also observed a partial substrate dependency of the inotropic effect. The maximal inotropic response to insulin was 50% smaller in pyruvate-containing Tyrode’s solution than in glucose-containing Tyrode’s solution at the higher insulin concentration. In addition, blocking glycolysis in muscles preincubated with glucose attenuated the inotropic response to insulin to the extent seen in the presence of pyruvate. We therefore suggest that the functional response to insulin may be related to 2 components: one that originates from improved glucose utilization and metabolism, and one that is independent from metabolic factors. However, we are not aware to what extent we have reduced glucose metabolism at the iodoacetate concentration used in these experiments, because higher concentrations of the inhibitor induced irreversible contracture of the muscles. Furthermore, basal developed force was significantly higher in the pyruvate group. This is in line with the described positive inotropic effect of pyruvate. With the higher basal twitch tension, an additional positive inotropic response may appear smaller if calculated as a percentage of basal force. In fact, the difference in the average increase in twitch force with 3 IU/L insulin was also less pronounced if absolute values were calculated: force increased by 4.3 mN/mm² in glucose-containing solution and by 3.3 mN/mm² in pyruvate-containing solution.

Signal Transduction Pathways of Insulin in Human Myocardium
In addition to binding to sarcolemmal insulin receptors, insulin is able to bind to both types of IGF receptors.
receptors results in a Ca\textsuperscript{2+}-dependent positive inotropic effect.\textsuperscript{22} Nevertheless, in the present study, insulin effects were not significantly blunted by preincubation with the selective IGF-1 receptor antibody α-IR-3. This demonstrates an IGF receptor–independent signal transduction pathway for functional effects of insulin in human myocardium, most likely via direct activation of the insulin receptors. Both insulin receptors and IGF-1 receptors initiate signal transduction pathways that involve the insulin-receptor substrate (IRS). Tyrosine-phosphorylated IRS consecutively activates signaling molecules with SH2 domains, including PI-3-kinase.\textsuperscript{34,35}

The PI-3-kinase–dependent pathway mediates antiapoptotic signaling and trophic effects via activation of Akt. The role of PI-3-kinase activation in mediating metabolic actions of insulin is controversial.\textsuperscript{36–38} In the present study, preincubation of human ventricular muscle with the selective PI-3-kinase inhibitor wortmannin almost completely prevented the insulin-dependent inotropic effects. Similarly, we could previously demonstrate that PI-3-kinase also mediates IGF-1–dependent functional effects.\textsuperscript{22} These data suggest that PI-3-kinase activation is a key event in the signal transduction network activated by insulin or IGF-1 that ultimately results in functional effects. We also tested the involvement of other kinases, such as PKC, which may be activated downstream of PI-3-kinase\textsuperscript{39}; however, neither inhibition of PKC nor inhibition of DAG had any measurable effect on the inotropic response of failing human cardiac muscle to insulin. Therefore, the chain of events that couples insulin receptors and PI-3-kinase activation to functional effects deserves further investigation.

**Influence of Insulin on Intracellular Ca\textsuperscript{2+} Handling**

An increase in [Ca\textsuperscript{2+}], as the underlying mechanism for the inotropic effect of insulin was demonstrated for rat whole-heart preparations.\textsuperscript{3} Therefore, we assessed the effects of insulin on intracellular Ca\textsuperscript{2+} handling in human cardiac muscle. We could demonstrate that intracellular Ca\textsuperscript{2+} transients increased in parallel to twitch force in aequorin-loaded trabeculae. Increased Ca\textsuperscript{2+} transients may result from increased transsarcolemmal Ca\textsuperscript{2+} influx, increased SR Ca\textsuperscript{2+} release, or both. We therefore directly tested the effects of insulin on SR Ca\textsuperscript{2+} content and SR Ca\textsuperscript{2+} reuptake via SERCA2a (relative to transsarcolemmal Ca\textsuperscript{2+} elimination) using RCCs. We could demonstrate that the inotropic response to insulin was associated with but not dependent on an increase in SR Ca\textsuperscript{2+} content. In addition, data from the paired rapid cooling experiments revealed that insulin did not change the relative contribution of SERCA2a versus Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange for cytosolic Ca\textsuperscript{2+} elimination. This supports the hypothesis that insulin increases transsarcolemmal Ca\textsuperscript{2+} influx without directly affecting SR Ca\textsuperscript{2+} handling properties. It also supports our notion that substantial parts of the inotropic effects of insulin are not related to metabolic changes, which might (by improved phosphorylation potential) increase the Ca\textsuperscript{2+}-reuptake capacity of SERCA2a.\textsuperscript{40}

PI-3-kinase–dependent activation of reverse-mode Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange recently has been shown by our group to be one source of Ca\textsuperscript{2+} entry in human myocardium.\textsuperscript{22} Therefore, we directly tested the effects of pharmacological reverse-
pects of insulin signaling in the human heart deserve further investigation.

Study Limitations
A limitation of the study is that aequorin light signals have not been converted to [Ca2+]i. This in itself is not a problem, but it reduces the validity of direct comparisons between different inotropic interventions. Although validated for human cardiac muscle, rapid cooling may not release all Ca2+ stored within the SR. In addition, RCCs are an indirect measure of SR Ca2+ content, and subcellular changes, such as the insulin-dependent increase in myofilament responsiveness to Ca2+, may affect RCCs. KB-R7943 was used as an inhibitor of the Na+/Ca2+ exchanger reverse mode; however, KB-R7943 is not an ideal agent because it can also affect other transport systems, such as K+, Na+, and Ca2+ channels, and it affects Ca2+ transients even in Na+/Ca2+ exchanger–knockout heart tubes. The apparent selectivity for outward versus inward Na+/Ca2+ exchanger current is not well understood. Nevertheless, no better reverse-mode inhibitors are available presently.

Acknowledgment
This work was supported by grants from the Deutsche Forschungsgemeinschaft (DFG Pi-414/1 and DFG Pi-414/2), as well as the German Federal Ministry of Education and Research (BMBF; Competence Network Heart Failure, TP8, Basic Mechanisms) to Dr Pieske.

References


45. Reuter H, Henderson SA, Han T, Matsuda T, Baba A, Ross RS, Goldhaber JJ, Philipson KD. Knockout mice for pharmacological screening: testing the specificity of Na\textsuperscript{+}/H\textsuperscript{+}-Ca\textsuperscript{2+}/H\textsuperscript{+} exchange inhibitors. *Circ Res*. 2002;91:90–92.


Insulin Causes [Ca\textsuperscript{2+}]\textsubscript{i}-Dependent and [Ca\textsuperscript{2+}]\textsubscript{i}-Independent Positive Inotropic Effects in Failing Human Myocardium

Dirk von Lewinski, Sebastian Bruns, Stefanie Walther, Harald Kögler and Burkert Pieske

_Circulation_. 2005;111:2588-2595; originally published online May 9, 2005;
doi: 10.1161/CIRCULATIONAHA.104.497461

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/111/20/2588

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the _Permissions and Rights Question and Answer_ document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/