How Does Glucose Insulin Potassium Improve Hemodynamic Performance?
Evidence for Altered Expression of Beta-Adrenoreceptor and Calcium Handling Genes

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Background—Glucose insulin potassium (GIK) improves hemodynamic performance after coronary artery surgery (CABG). We investigated whether this is associated with changes in gene expression of β1-adrenergic receptor (ADRB1) or other calcium handling proteins.

Methods and Results—During a randomized double-blind placebo-controlled trial, 48 patients undergoing on-pump CABG, allocated to receive pre-ischemic placebo (5% dextrose) or GIK (40% dextrose, K+/H11001 100 mmol.L⁻¹, insulin 70 u.L⁻¹; 0.75 mL.kg⁻¹.h⁻¹) continued for 6 hours after the removal of the aortic cross-clamp (AXC), underwent left ventricular biopsy for analysis of specific mRNAs immediately before AXC, before release of AXC, and 10 minutes after reperfusion (placebo n=24, GIK n=24). GIK or placebo was infused for a mean of 79±21 minutes or 79±18 minutes pre-ischemia respectively. Serial hemodynamic measurements were performed. Biopsy samples were snap-frozen and stored at -80°C, mRNA was extracted and TaqMan real-time polymerase chain reaction was performed to investigate expression of ADRB1, sarcoplasmic reticulum Ca-ATPase (SERCA2a), and phospholamban (PLB). GIK significantly increased cardiac index versus placebo (P=0.037). TaqMan reverse-transcriptase polymerase chain reaction showed significantly greater ADRB1 mRNA expression at all time points (4.9-fold, 7.4-fold, and 15.6-fold increase, respectively; P<0.001), significantly greater SERCA2a mRNA expression after reperfusion (13.2-fold; P<0.001), and increased PLB mRNA expression at pre-ischemia and reperfusion (P<0.001 for both time-points) in GIK groups versus placebo.

Conclusions—The beneficial hemodynamic effects of GIK therapy are associated with increased ADRB1 and SERCA2a mRNA expression. Further work is therefore warranted to investigate these mRNA effects at the protein level. (Circulation. 2006;114[ suppl I]:I-239–I-244.)

Key Words: beta-adrenergic receptors ■ calcium ■ glucose ■ hemodynamics ■ sarcoplasmic reticulum

Glucose insulin potassium administration (GIK) is considered an adjunct to myocardial protection during ischemia and reperfusion. In cardiac surgery, GIK improves cardiovascular function and reduces inotropic requirement and myocardial injury after coronary artery bypass surgery (CABG).¹ The mechanisms of action of GIK, although ill-understood, probably relate to both glucose and insulin. Glucose is the more efficient and preferred substrate of the ischemic myocyte. Insulin is a vasodilator² and inotropic,³,⁴ reduces circulating free fatty acids,¹ promotes glucose uptake,⁵ and reduces myocardial apoptotic injury when administered at reperfusion.⁶ Insulin also increases β-adrenergic receptor sensitivity and calcium handling properties of the myocyte.⁷,⁸ In this study, we examined the effects of peri-ischemic GIK on changes in the expression of mRNAs encoding β1-adrenergic receptors (ADRB1), sarcoplasmic reticulum calcium ATPase (SERCA), and phospholamban (PLB) in the left ventricle (LV), and their relationship to hemodynamic function after CABG.

Methods

Study Design
During 2 consecutive randomized double-blind prospective placebo controlled trials, 317 adult nondiabetic patients undergoing isolated CABG were randomized to receive either placebo (n=160) or GIK (n=157). From the study populations, a consecutive series of the most recent 48 patients (placebo n=24 and GIK n=24) underwent serial perioperative LV biopsy for analysis of ADRB1, SERCA (2a

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isofrom), and PLB messenger RNA (mRNA) expression. The Local Research Ethics Committee approved the studies and all patients gave written informed consent. Patients were randomized via a computer-generated schedule. All trial investigators, including mRNA analysts, were blinded to allocation.

GIK infusions consisted of 40% dextrose, K+ 100 mmol·L⁻¹, insulin 70 IU·L⁻¹ (Human Actrapid Insulin; Novo Nordisk A/S, Bagsvaerd, Denmark). Placebo therapy was 5% dextrose. GIK/placebo therapy was administered from operation start time until 6 hours after removal of aortic cross-clamp (AXC) as a central intravenous infusion at 0.75 mL·kg⁻¹·h⁻¹. Anesthesia, cardiopulmonary bypass (28°C), and surgical techniques were standardized.¹

Preoperative clinical details, operative details, and postoperative details are described.⁹

RNA Extraction and Reverse Transcription

Total RNA was extracted from LV biopsy samples using the Sigma Trisol kit, a single-step acid guanidinium phenol-chloroform extraction procedure, following the manufacturer’s guidelines. After dissolution in ethanol, aliquots were transferred to RNAqueous96 96-well extraction system plates (Ambion, Austin, Tex), and RNA was extracted according to the manufacturer’s guidelines. RNA was reverse-transcribed using avian myeloblastosis virus reverse-transcriptase (Promega, Madison, Wis) in a total reaction volume of 20 μL, with 1 μL of total RNA, 30 pmol random hexamer primers, 4 μL of 5x avian myeloblastosis virus reverse-transcriptase buffer, 2 μL of deoxynucleotide triphosphate (dNTP) mix (200 μmol·L⁻¹ each), 20 U of ribonuclease inhibitor (RNasin; Promega), and 15 U of avian myeloblastosis virus reverse-transcriptase (Promega), as previously published⁹ and converted to epinephrine once dopamine 10 μg·kg⁻¹·h⁻¹ was reached.

Quantitative Polymerase Chain Reaction

Expression of specific mRNAs was determined using the ABI PRISM 7700 sequence detection system. Reverse-transcriptase polymerase chain reaction was performed in 25-μL volumes on 96-well plates, in a reaction buffer containing 1x TaqMan Universal PCR Master Mix, 0.75 μL TaqMan probe per reaction, and 4.5 μL forward and reverse primer per reaction, as we have described previously.⁹,¹⁰ All reactions were multiplexed with a pre-optimized control probe for 18s RNA (ABI, Warrington, UK). Primer and probe sequences are given in Table 1. As per the manufacturer’s guidelines, data were expressed as cycle threshold (Ct) values and used to determine ΔCt values (Ct = Ct of the target gene [eg, ADRB1] minus Ct of the housekeeping gene). To exclude potential bias caused by averaging data that had been transformed through the equation 2⁻ΔΔCt to give fold changes in gene expression, all statistics were performed with ΔCt values, as described previously.⁹ Target gene probes were labeled with FAM, and the housekeeping gene with VIC. Reactions were as follows: 50°C for 2 minutes and 95°C for 10 minutes and then 40 cycles. All reverse-transcriptase polymerase chain reaction assays were repeated a minimum of 3 times. Only samples demonstrating consistent findings were used in subsequent analyses.

Western Protocol

Whole-cell protein extracts were prepared from LV biopsy samples in lysis buffer (100 mmol/L sodium chloride, 0.1% Triton X-100, and 50 mmol/L Tris [pH 8.3]) containing enzyme inhibitors. Protein concentration before loading was measured by the Bradford assay with bovine serum albumin as standard. Soluble proteins (30 μg) were separated by electrophoresis in 12.5% sodium dodecyl sulfate polyacrylamide gels, transferred to polyvinylidene fluoride membranes, incubated in 5% nonfat milk in phosphate-buffered saline with 0.1% Tween, followed by incubation with primary antibodies SERCA2a (mouse monoclonal; Abcam, Cambridge, UK; 1:1000 dilution); Phospholamban (mouse monoclonal; Abcam; 1:500); β1 adrenergic receptor (Rabbit polyclonal; Santa Cruz, Calif; 1:100). After washing in phosphate-buffered saline plus 0.1% Tween, blots were incubated with appropriate secondary antibodies conjugated to horseradish peroxidase. After additional washes, antigen–antibody complexes were visualized by the ECL chemiluminescence detection system (Amersham Biosciences, Buckinghamshire, UK).⁹

The authors had full access to the data and take responsibility for their integrity. All authors have read and agree to the manuscript as written.

Statistical Analysis

Data were analyzed using SPSS version 12.0 software (SPSS Inc.). Categorical data were compared using χ² testing. Continuous data are presented as mean and standard deviation or median and interquartile range. Normally distributed data were compared using an independent t test. Skewed data were analyzed nonparametrically (Mann-Whitney U test) as appropriate. Serial measurements were compared by repeated measures analysis of variance (ANOVA). Statistical significance was assigned when P≤0.05.

Results

Preoperative clinical details, operative details, and postoperative details are described in Table 2 (no significant differences between placebo and GIK subjects). Patients received GIK infusions for 78±21 minutes and placebo for 78±18 minutes before the initial biopsies. There were no significant differences in the preoperative ejection fraction, New York Heart Association score, or urgency of surgery between the 2 groups.

Hemodynamic Outcomes and Inotrope Usage

In the reported study population (n=48) those patients receiving GIK therapy demonstrated a significant increase in cardiac index during the first 6 hours after removal of the AXC (P=0.037) compared with placebo (Figure 1). This

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**TABLE 1.** Primer and Probe Sequences for Taqman Real-Time Polymerase Chain Reaction mRNA Analysis of β1-Adrenergic Receptor (ADRB1), Sarcoembrilasmic Reticulum Calcium ATPase 2a (SERCA 2a), and Phospholamban (PLB)

<table>
<thead>
<tr>
<th>ACT Number</th>
<th>Primer Sequence 1</th>
<th>Primer Sequence 2</th>
<th>Primer Sequence 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADRB1</td>
<td>5′ TCTAGTGGCCCGCTGGTAC 3′</td>
<td>5′ TGATCTTCTTTACAGTGTT 3′</td>
<td>5′ CTTCCGGTGACCTGCGGTTCT 3′</td>
</tr>
<tr>
<td>SERCA 2a</td>
<td>5′ GCAAAGACGGAACTCTGGAAG 3′</td>
<td>5′ ACAAAAGCGAGAATCCTC 3′</td>
<td>5′ CCCGTGCTTGGCGGCTGAC 3′</td>
</tr>
<tr>
<td>PLB</td>
<td>5′ TCAAGCTGATAGAAGGCTCAACCA 3′</td>
<td>5′ GATGAGAGACAAATGATAATGATTGCTG 3′</td>
<td>5′ GAGAACCGCAAAAGGACCTCAACCA 3′</td>
</tr>
</tbody>
</table>
effect was not maintained following cessation of GIK therapy in the 6- to 12-hour period after AXC removal. During the postoperative period there was a reduced need for inotropic support in the GIK group (Table 3).

**TABLE 3. Inotropic Support in the First 12 Hours After Aortic Cross-Clamp (AXC) Removal**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>GIK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine use 0–6 hours after AXC removal</td>
<td>12 (50)</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Dopamine use 6–12 hours after AXC removal</td>
<td>14 (58.3)</td>
<td>6 (25)</td>
</tr>
<tr>
<td>Epinephrine use 0–6 hours after AXC removal</td>
<td>2 (8.3)</td>
<td>—</td>
</tr>
<tr>
<td>Epinephrine use 6–12 hours after AXC removal</td>
<td>3 (12.5)</td>
<td>2 (8.7)</td>
</tr>
</tbody>
</table>

Data are shown as N (%).

**Figure 1.** Effect of peri-ischemic glucose insulin potassium (GIK) infusion on cardiac index (CI), $P=0.037$ for first 6 hours after aortic cross clamp (AXC) removal.

**Phospholamban Expression**

Because PLB regulates SERCA activity, PLB expression was also studied (Figure 4). A small increase in PLB mRNA expression was noted in the placebo group over time (1.4- and 1.8-fold, at ischemia and reperfusion, respectively). However, administration of GIK led to marginal reductions in the amount of PLB mRNA expressed over time. Comparison with placebo showed that GIK administration led to 3.2- and 2.4-fold increases in PLB expression at pre-ischemia and reperfusion ($P<0.001$).

**Western Blots**

We also sought to determine whether ADRB1, SERCA 2a, and PLB proteins were detectable in our biopsies of human heart (Figure 5). Although no attempt was made to quantify protein expression because of limitations of biopsy size and the early time points investigated, all 3 gene products were readily detectable by Western blotting.

**Discussion**

We have demonstrated that peri-ischemic GIK administration during CABG leads to increased myocardial expression of the mRNAs encoding ADRB1, SERCA 2a, and PLB. These findings are associated with improved post-operative hemo-
dynamic function but are unlikely to represent the sole mechanism for GIK’s hemodynamic effects. GIK also improves substrate utilization, reduces myocardial cellular damage and reduces afterload.\textsuperscript{1,2,11} Some, but not all, published studies of GIK in cardiac surgery demonstrate hemodynamic benefit. Lack of consistency may reflect differing GIK regimens, different times and lengths of administration of therapy, and the use of different outcome measures.\textsuperscript{1,11–13}

Our studies revealed an increase in ADRB1 mRNA in the LV after \( \approx 80 \) minutes of pre-ischemic GIK administration, with further increases after the stimuli of ischemia and reperfusion. Increase in PLB expression was observed before ischemia and after reperfusion, and an increase in SERCA 2a mRNA was seen after reperfusion. These findings in gene expression observed at the mRNA level are likely to result in subsequent protein changes, which may contribute to the hemodynamic effects of GIK during CABG.

In addition to its primary role in glycemic control, insulin is known to have a wide variety of genomic effects, mediated by specific receptors.\textsuperscript{5} The mechanism by which insulin modulates myocardial contractility via the \( \beta \)-adrenergic system is ill-understood. In heart failure, there is a reduced inotropic response to \( \beta \)-adrenergic stimulation and a decrease in the number of \( \beta \)-adrenergceptors.\textsuperscript{14–16} Insulin may also improve contractility by a calcium-independent sensitization of ADRB1.\textsuperscript{7} Published data have demonstrated that levels of ADRB1 mRNA change in response to periods of ischemia\textsuperscript{17} and that the regulation of \( \beta \)-adrenergic receptors predominantly reflects changes in mRNA expression.\textsuperscript{18} We have confirmed that increased ADRB1 mRNA expression occurs after ischemia and reperfusion and that peri-ischemic GIK significantly augments this effect at all time points. Thus, there are 2 \( \beta \)-1-receptor effects caused by insulin. Sensitization of the functional receptor and secondly, a likely increase in receptor number secondary to increased pretranslational expression.

The sarcoplasmic reticulum sequesters calcium and subsequently releases it into the sarcoplasm to initiate contraction. Sequestration is brought about as an active process by the SERCA family of proteins, of which SERCA2a is the predominant isoform in cardiac muscle.\textsuperscript{19} SERCA acts by actively transporting calcium into the sarcoplasmic reticulum after contraction, leading to diastolic relaxation. Increased SERCA2a activity within the myocardium leads to enhanced diastolic relaxation and improved contraction as more calcium is consequently available to be released from the
sarcoplasmic reticulum. Insulin has previously been demonstrated to increase expression of SERCA at both an mRNA and protein level within vascular smooth muscle cells.20 In isolated myocytes from failing human hearts, insulin leads to both calcium-dependent and calcium-independent positive inotropy.8 When glucose is used as the substrate for such myocytes, the positive inotropic effects of insulin are concentration-dependent. These inotropic effects are associated with an increase in sarcoplasmic reticulum calcium content.8 Our finding that GIK increases post-reperfusion SERCA2a mRNA expression may in part explain this observation of increased sarcoplasmic reticulum calcium content. If this change in mRNA translates to changes at a protein level it provides a further mechanism by which GIK may act as a positive inotrope. The time frame in which we observed changes in SERCA2a expression are short, but it has been demonstrated that direct transcriptional regulation of other genes can occur within this period.21

PLB is the principal regulator of SERCA within the myocyte. In response to ADRB1 stimulation, PLB is phosphorylated.22 This action increases the sensitivity of SERCA to calcium and results in increased calcium uptake into the sarcoplasmic reticulum with a subsequent improvement in diastolic relaxation and increase in force of contraction. PLB is believed to be modulated by SERCA in 2 separate ways, the first being a short-acting mechanism related to phosphorylation state, and the second a long-acting mechanism via increased expression of SERCA and reduction in expression of PLB. This response has been demonstrated in rodent studies.23 Although we have shown in our present study that PLB mRNA expression is increased for GIK over placebo we have not investigated the phosphorylation status of PLB. The relevance of the observed changes in PLB expression are thus more difficult to predict than those for ADRB1 and SERCA.

In conjunction with the changes observed in the mRNA expression of ADRB1, SERCA2a, and PLB, we have demonstrated the presence of these proteins in Western blots. We did not explore differences in protein expression because of the relatively short time period between administration of GIK and biopsies. The timing of biopsies after reperfusion was constrained by the need to limit operation time and patient risk. However, all 3 proteins were expressed at readily detectable levels in our samples.

The observed changes in expression of several genes relevant to myocardial function are associated with improved hemodynamic performance at later time points. This hemodynamic change is likely to be induced by a range of possible mechanisms and the relative importance of these has yet to be elucidated.11 However, if the changes in ADRB1 expression and the expression of genes determining the calcium handling properties of the myocyte are paralleled at a protein level, this mechanism may play a crucial role in producing the observed changes in cardiovascular performance generated by peri-ischemic GIK administration.

Limitations
We demonstrated improved hemodynamic performance for this consecutive cohort of subjects who underwent LV biopsy concordant with the effects observed in the larger study population.1 Although a specific inotropic effect of insulin has demonstrated experimentally,4 we cannot be sure that contractility improved in this clinical study as load-independent measures were not used. Second, although we have demonstrated changes in mRNA expression we have only analyzed these end points in a small cohort. Another limitation of our study is that we have demonstrated differences in gene expression at an mRNA level, but because of limitations in biopsy size and the time points of sampling we did not investigate directly changes in protein expression or function.

Acknowledgments
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Disclosures

None.

References


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