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Glucose-transporter–mediated positive inotropic effects in human myocardium of diabetic and nondiabetic patients

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Abstract

Insulin causes inotropic effects via Ca^{2+}-dependent and Ca^{2+}-independent pathways. The latter one is potentially glucose dependent. We examined inotropic responses and signal transduction of insulin in human atrial myocardium of diabetic and nondiabetic patients to test for the role of glucose transporters. Experiments were performed in isolated atrial myocardium of 88 patients undergoing cardiac surgery and 28 ventricular muscle samples of explanted hearts. Influence of insulin (0.02 μmol/L) on isometric twitch force was examined with and without blocking glucose transporter (GLUT) 4 translocation (latrunculin), sodium-coupled glucose transporter (SGLT) 1 (phlorizin, T-1095A), or PI3-kinase (wortmannin). Experiments were performed in Tyrode solution containing glucose or pyruvate as energetic substrate. Messenger RNA expression of glucose transporters (GLUT1, GLUT4, SGLT1, SGLT2) was analyzed in atrial and ventricular myocardium of both diabetic and nondiabetic patients. Developed force increases after insulin (to 117.8% ± 2.4% and 115.8% ± 1.9%) in trabeculae from patients with and without diabetes. Inotropic effect was reduced after displacing glucose with pyruvate as well as after PI3-kinase inhibition (to 103% ± 2%) or inhibition of glucose transporters GLUT4 (to 105% ± 2%) and SGLT1 (phlorizin to 106% ± 2%, T-1095A to 105% ± 2%), without differences between the 2 groups. In glucose-free pyruvate-containing solution, only inhibition of PI3-kinase but not blocking glucose transporters resulted in further inhibitory effects. Messenger RNA expression did not show significant differences between patients with or without diabetes. Insulin exerts positive inotropic effects in human atrial myocardium. These effects are mediated via a PI3-kinase–sensitive and a glucose-transport–sensitive pathway. Differences in functional effects or messenger RNA expression of glucose transporters were not detectable between patients with and without diabetes.

1. Introduction

Diabetes is a major risk factor for cardiovascular morbidity, and patients with chronic heart disease often suffer from insulin resistance, diabetes, or altered glucose metabolism [1,2]. The onset of insulin resistance has been shown to coincide with the transition of hypertrophy to dilatation [3]. Whole-body insulin resistance is prevalent in congestive heart failure patients with either ischemic heart failure or idiopathic dilated cardiomyopathy [4,5].

According to a recent analysis from the Framingham Heart Study, the proportion of cardiovascular disease attributable to diabetes has substantially increased over the past decades [6].

Besides its known metabolic characteristics, insulin exerts functional effects in myocardial tissue, that is, inotropic effects. These functional effects of insulin have been tested in many models, leaving controversial data of either positive inotropic effects [7-13] or no functional effects [14-16]. Differences in functional effects between control and diabetic animals have been described in various models including lamb [17], rats [9,18], and humans [10,19]. Nevertheless, we have not observed significant differences on developed force in a small substudy in failing human myocardium [13].

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In our recent work, we have noted pronounced positive inotropic effects of insulin in ventricular muscle strips of terminally failing human hearts. It has been shown that the positive inotropic effect is partially Ca^{2+} dependent and in other parts glucose dependent as shown in glucose-free solution and after pharmacologic inhibition of glycolysis.

Underlying mechanisms of the glucose-dependent-mechanism, however, remained unclear; and a role for glucose transporters appears to be likely.

Glucose transporters are divided into 2 major families: The large glucose transport facilitator (GLUT) family and the sodium-coupled glucose transporter (SGLT) family. Within the former group, GLUT4 is the most common transporter directly regulated by insulin due to initiation of a phosphorylation cascade that stimulates the translocation of GLUT4 from intracellular membranes to the plasma membrane, resulting in an immediate 10- to 20-fold increase in glucose transport. GLUT4 accounts for two thirds of GLUT messenger RNA (mRNA) detected in fetal human hearts; the other third is GLUT1 mRNA. The ratio is shifted toward GLUT4 in adult hearts and even further in failing human hearts [20]. Recently, SGLT1 mRNA has also been reported in human myocardium [21]. This glucose transporter is mainly located in the small intestine and kidneys, reabsorbing glucose, and is undetectable in myocardium of most animal species. However, Zhou et al [21] have detected high quantities of mRNA in human cardiomyocytes and verified their finding by in situ hybridization using labeled antisense complementary RNA probes.

No data are available on functional effects of insulin in atrial human muscle strip preparations in general and diabetic hearts specifically. Moreover, first functional data on GLUT4 in a GLUT4 knockout and knockdown model have only just recently been published, indicating a direct link between genomic and functional effects [22]. To date, no data on functional effects of SGLT1 are available.

Because heart transplantation is rare in diabetic patients and therefore ventricular tissue of diabetic patients is not readily accessible, atrial myocardium is suited better to test for differences between diabetic and nondiabetic human myocardium. Therefore, we directly assessed functional effects of insulin and its dependence on glucose transport in isolated atrial human myocardium. We compared functional effects as well as glucose transporter mRNA expression in diabetic and nondiabetic patients.

2. Materials and methods

2.1. Human myocardium

Experiments were performed in atrial muscle strips isolated from right atrial appendages from 88 hearts. Twenty-five patients required valve replacement; 54, bypass surgery; and 9 patients, combined bypass and valve replacement. Sixty-five patients were male. A small (5-8 mm × 5-8 mm) piece was excised from the right atrium before cannulation and the administration of the cardioplegic solution.

Functional experiments were performed in muscle strips of 61 patients. Average ejection fraction was 56.1% ± 2.6% (14 patients with an ejection fraction <55%). The mean age of the patients was 69.7 ± 1.2 years. Average body mass index was 28.2 ± 1.5. Coronary heart disease was present in 52 patients. Medication included angiotensin-converting enzyme inhibitors in 49 patients, β-blockers in 36 patients, and diuretics in 31 patients.

Expression experiments were performed in 27 atrial and 28 ventricular shock-frozen tissue samples. Atrial tissue originated from patients undergoing cardiac surgery. Average ejection fraction was 52% ± 3.1% (11 patients with an ejection fraction <55%). The mean age of the patients was 69.1 ± 1.1 years. Average body mass index was 29 ± 1.6. Coronary heart disease was present in 23 patients. Medication included angiotensin-converting enzyme inhibitors in 20 patients, β-blockers in 10 patients, and diuretics in 15 patients.

Ventricular tissue samples originated from 28 end-stage failing hearts (18 of ischemic, 10 of dilative etiology; 26 male and 2 female) during heart transplantation. The mean age of the patients was 61.1 ± 1.6 years. The mean ejection fraction was 22.3% ± 1.6%; mean cardiac index was 1.9 ± 0.1 L/(min m^2). Patients were divided into a group of 13 patients with preexisting diabetes (3 patients insulin dependent, 10 patients on oral medication) and 15 nondiabetic control patients. Average age was 59.5 ± 1.5 and 63.1 ± 0.9 years, respectively (not significant, $P = .074$). Ischemic cardiomyopathy was present in 10 patients of the diabetes group and 8 patients of the control group. Ventricular myocardium was only used for mRNA analysis.

This study was approved by the Hospital Ethics Committee, and all patients gave written informed consent to participate in the study.

2.2. Muscle strip preparation

Appendages were transported to the laboratory in ice-cold cardioplegic Tyrode solution containing (in millimoles per liter): Na^+ 152, K^+ 3.6, Cl^- 135, HCO_3^- 25, Mg^{2+} 0.6, $H_2PO_4^-$ 1.3, SO_4^{2-} 0.6, Ca^{2+} 0.2, glucose 11.2, and 2,3-butanedione-monoxime 30, equilibrated with carbogen (95% O_2, 5% CO_2) to a pH of 7.4. This solution has been shown to protect the myocardium during transportation and from cutting injury at the time of dissection, with full reversibility of the cardioplegic effects upon washout. Small endocardial trabeculae ("muscle strips," cross-sectional area <0.5 mm^2) were dissected with the help of a stereomicroscope.

Small muscle strips (n = 140) were dissected and mounted in special chambers between miniature hooks, connected to an isometric force transducer (Scientific Instruments, Heidelberg, Germany), and superfused with modified Tyrode solution (37°C) of the composition given above except that 2,3-butanedione-monoxime was omitted and $[Ca^{2+}]_o$ was stepwise increased to 2.5 mmol/L [23].

Muscles were electrically stimulated at 1 Hz (37°C), and isometric contractions were recorded at optimum preload (L_{max}). In a subset of experiments, glucose was replaced, as indicated, by 22.4 mmol/L pyruvate as energetic substrate.

2.3. Drugs

Insulin (Sigma Chemicals, Munich, Germany) was dissolved in distilled water to a stock solution of 10 mmol/L. Twenty microliters of the stock solution was added to the bath (100 mL) to achieve a final concentration of 20 nmol/L. T-1095A (1 μmol/L; gift of Merck KGaA, Darmstadt, Germany), latrunculin B (0.1 μmol/L), wortmannin (0.1 μmol/L), and phlorizin (200 μmol/L) (all Sigma Chemicals) were added to the organ bath 30 minutes before the experiment.

2.4. mRNA expression

2.4.1. SGLT

Total RNA was isolated using a total RNA isolation kit (Qiagen, Hilden, Germany). Quality and quantity of RNA were assessed using spectrophotometry and using Agilent Bioanalyzer (Böblingen, Germany). RNA was transcribed into complementary DNA (cDNA) using oligo dT Primers and a reverse transcriptase polymerase chain reaction kit (Roche Diagnostics, Mannheim, Germany). All samples were diluted to a starting concentration of 200 pg/μL. The copies of SGLT1 and SGLT2 mRNA genes were detected by measuring 5 μL of each diluted cDNA. Values were calculated and normalized against a standard and housekeeping gene. Expression of genes was detected using a designed FRET expression assay (TIB Molbiol, Berlin, Germany; assay no. 006111456 for SGLT1 and SGLT2) and was compared with the housekeeping gene RNA2 polymerase (TIB Molbiol, assay no. 005115452).

2.4.2. GLUT

Total RNA was extracted by the TRIZOL method (Invitrogen, Carlsbad, CA) and further purified using RNeasy Mini Kit (Qiagen). Quality and quantity of RNA were assessed using spectrophotometry and using Agilent Bioanalyzer. RNA was transcribed into cDNA. High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) and LightCycler (Roche) were used for reverse transcription. Expression of genes was detected using Taqman and was compared with the housekeeping gene glucose-6-phosphate dehydrogenase (G6PDH). Primers used for GLUT4 were 5′-GCTACCTCTACATCATCCAGAATCTC-3′ (forward), 5′-CCAGAAACATCGGCCCA-3′ (reverse), and TaqMan Probe 5′-FAM-CTGCCAGAAAGAGTCTGAAGCGCCT-TAMRA-3′ Primer (TIB Molbiol).

GLUT1 was tested using the commercial "TaqMan Gene Expression Assay Hs00892681_m1" (Applied Biosystems).

2.5. Statistical analysis

Data are expressed as mean ± SEM. Differences were compared by 2-way analysis of variance following Kolmogorov-Smirnov test and QQ plots for normal distribution and Levene test for homoscedasticity. Statistical significance was taken as $P < .05$. The mRNA data were tested for normal distribution using Shapiro-Wilk test, and Wilcoxon test was used in groups without normal distribution of values.

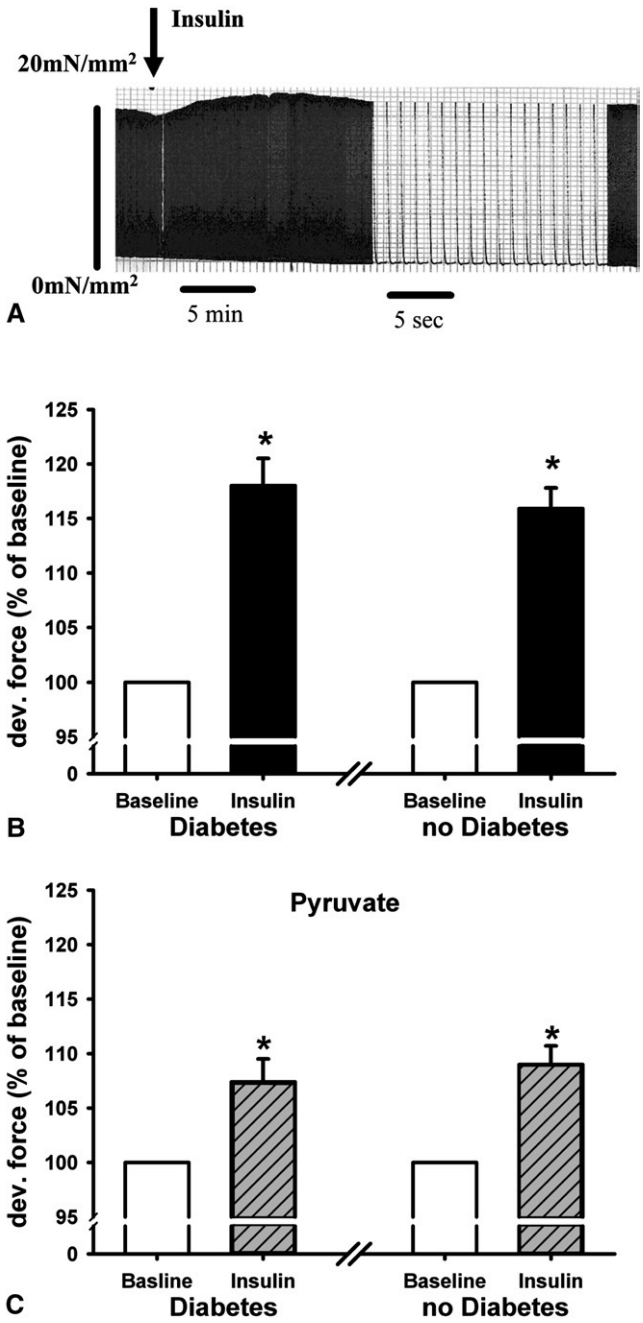

Fig. 1. A, Upper panel: effect of 0.02 μmol/L insulin on isometric twitch tension in an atrial muscle strip of a human heart. B, Effect of insulin (0.02 μmol/L) in myocardial muscle strips of patients with diabetes (left) or without diabetes (right). Energetic substrate in the solution is 11.2 mmol/L glucose. *$P < .05$ vs control. C, Effect of insulin (0.02 μmol/L) in myocardial muscle strips of patients with diabetes (left) or without diabetes (right). Energetic substrate in the solution is 22.4 mmol/L pyruvate. *$P < .05$ vs control.

3. Results

3.1. Positive inotropic effects of Insulin

Insulin exerted positive inotropic effects in isolated human atrial muscle (Fig. 1A) without changes in diastolic tension or twitch kinetics. These functional effects of insulin were comparable in muscle strips from patients with (n = 23) and without diabetes (n = 27) with maximum increases in developed force to 117.8% ± 2.4% and 115.8% ± 1.9% of baseline value, respectively (both *P*s < .05, Fig. 1B).

In pyruvate-containing solution, positive inotropy was reduced to 107.4% ± 2.2% (n = 12) and 109% ± 1.7% (n = 13) in diabetic and nondiabetic patients, respectively (Fig. 1C). Developed force at baseline conditions tended to be higher in the pyruvate groups with 28.6 ± 3.1 vs 24.2 ± 2.2 mN/mm^2 (*P* > .05). Insulin did not affect twitch kinetics under any of the described conditions.

3.2. Subcellular mechanisms of action of insulin

Preincubation of muscle strips with the PI3-kinase inhibitor wortmannin (0.1 μmol/L) reduced the inotropic response after insulin administration from 121.7% ± 4.3% and 114.8% ± 3.1% to 105.1% ± 3.5% and 101% ± 1.6% of baseline value in diabetic and nondiabetic hearts, respectively (both *P*s < .05, Fig. 2A). In pyruvate-containing Tyrode solution, insulin-mediated inotropy was even further blunted from 106.7% ± 2.6% to 100.1% ± 2.6% and from 109.9% ± 3% to 97.1% ± 1.3% in diabetic and nondiabetic patients, respectively (both *P*s < .05, Fig. 2B). There was no significant inotropic response to insulin after preincubation with wortmannin.

Inhibition of glucose transport also resulted in partially blunted inotropic response to insulin. Blocking GLUT4 translocation using latrunculin B (0.1 μmol/L) reduced the positive inotropic effect of insulin from 118.9% ± 3.7% and 115.3% ± 2.6% to 105.7% ± 3.8% and 105.1% ± 2.3% of baseline value in diabetic and nondiabetic hearts, respectively (both *P*s < .05, Fig. 3A). In glucose-free pyruvate-containing solution, the inotropic effect of insulin was reduced in the control group but not further altered after latrunculin B administration (108% ± 2.8% to 106% ± 2% and 107.3% ± 1.9% to 104.4% ± 1.4% in diabetic and nondiabetic hearts, both not significant, Fig. 3B). There was no significant inotropic response to insulin after preincubation with latrunculin B.

Comparable results were seen using SGLT1 inhibitor T-1095A. Positive inotropic effect of insulin was significantly reduced in glucose-containing solution (to 116.1% ± 2.7% vs 105.4% ± 2.6% and to 118.2% ± 3.3% vs 102.1% ± 1.9% in diabetic and nondiabetic hearts, both *P*s < .05), whereas no further inhibitory effect of glucose transport inhibition could be seen in pyruvate-containing solution (Fig. 4A, B). These inhibitory effects of SGLT1 blockade were confirmed using phlorizin, with 119% ± 3% (−Phlor, n = 6) vs 106.8% ± 4.4% (+Phlor, n = 6) and 114.8% ± 2.2% (−Phlor, n = 6) vs 105.9% ± 2.7% (+Phlor, n = 7) in diabetic and nondiabetic hearts (both *P*s < .05) using glucose-containing solution. A significant positive inotropic effect of insulin after preincubation with T-1095A was only detectable in diabetic patients using glucose-containing solution.

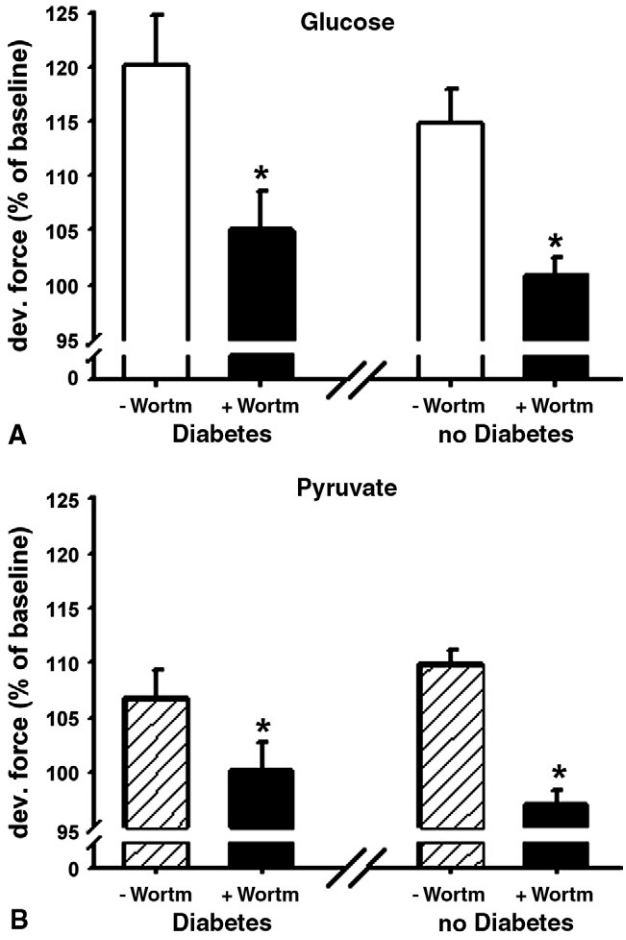

Fig. 2. A, Effect of insulin (0.02 μmol/L) in myocardial muscle strips with and without preincubation with PI3-kinase inhibitor wortmannin. Muscle strips from patients with diabetes (−Wort, n=7; +Wort, n=7; left) or without diabetes (−Wort, n = 9; +Wort, n = 9; right). Energetic substrate in the solution is 11.2 mmol/L glucose. Control muscle strips without wortmannin originate from the same hearts. **P* < .05 vs control. B, Effect of insulin (0.02 μmol/L) in myocardial muscle strips with and without preincubation with PI3-kinase inhibitor wortmannin. Muscle strips from patients with diabetes (−Wort, n = 10; +Wort, n = 10; left) or without diabetes (−Wort, n = 7; +Wort, n = 7; right). Energetic substrate in the solution is 22.4 mmol/L pyruvate. Control muscle strips originate from the same hearts. **P* < .05 vs control.

In a separate set of experiments, we analyzed the mRNA content of GLUT1 and GLUT4 as well as SGLT1 and SGLT2 in atrial and ventricular tissue samples of human hearts. As shown in Fig. 5, content of GLUT1 and GLUT4 was not affected either in atrium or in ventricle by coexisting diabetes mellitus. The relative expression of GLUT1 was (Fig. 5A) 1.0 ± 0.3 and 1.0 ± 0.14 in atrial and ventricular myocardium of patients with normal glucose metabolism and 0.72 ± 0.11 and 1.44 ± 0.30 in diabetic patients (not significant). Corresponding GLUT4 values were 1.0 ± 0.09

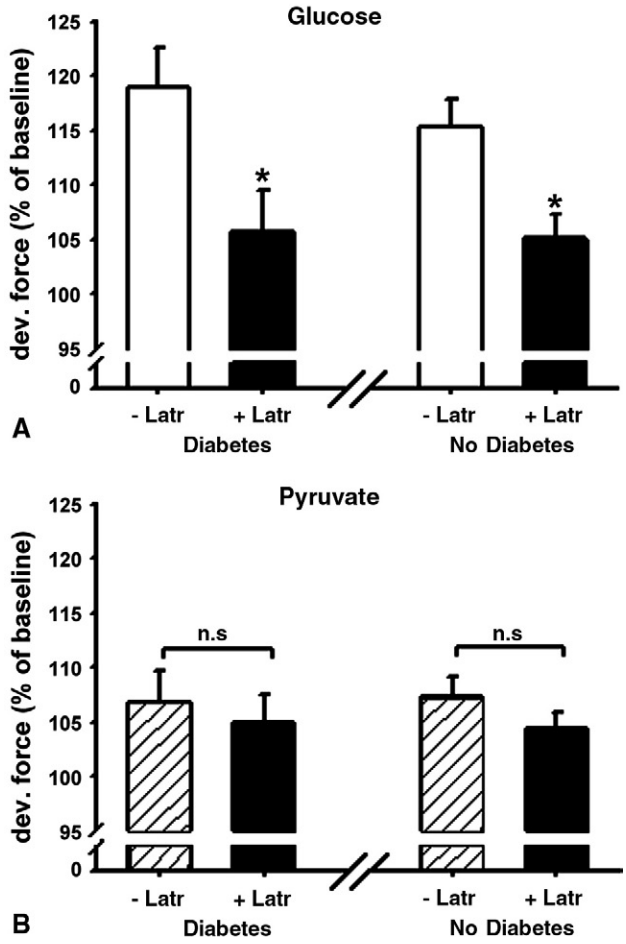

Fig. 3. A, Effect of insulin (0.02 μmol/L) in myocardial muscle strips with and without preincubation with GLUT4 translocation inhibitor latrunculin. Muscle strips from patients with diabetes (−Latr, n = 5; +Latr, n = 6; left) or without diabetes (−Latr, n = 11; +Latr, n = 10; right). Energetic substrate in the solution is 11.2 mmol/L glucose. Control muscle strips without preincubation with latrunculin originate from the same hearts. *P < .05 vs control. B, Effect of insulin (0.02 μmol/L) in myocardial muscle strips with and without preincubation with GLUT4 translocation inhibitor latrunculin. Muscle strips from patients with diabetes (−Latr, n = 8; +Latr, n = 8; left) or without diabetes (−Latr, n = 7; +Latr, n = 7; right). Energetic substrate in the solution is 22.4 mmol/L pyruvate. Control muscle strips originate from the same hearts. *P < .05 vs control.

and 1.0 ± 0.19 in atrial and ventricular myocardium of patients with normal glucose metabolism and 1.41 ± 0.35 and 0.79 ± 0.20 in diabetic patients (not significant). There were also no differences between patients with insulin-dependent diabetes mellitus and those on oral medication. Differences between diabetic and nondiabetic patients in content of housekeeping protein G6PDH could not be detected. SGLT1, too, did not show significant differences between the 2 groups (Fig. 5C); 13 500 ± 5100 copies per nanogram mRNA were detected in atrial myocardium of control patients vs 15 200 ± 4100 copies per nanogram mRNA in diabetic patients. In failing ventricular myocardium, corresponding values were 10 950 ± 3900 copies per nanogram mRNA in nondiabetic patients compared with 7850 ± 2600 copies per nanogram mRNA in diabetic hearts. In ventricular myocardium, patients with insulin-dependent diabetes mellitus (n = 3) tended to have higher expression of SGLT1 compared with patients on oral antidiabetic medication (10 200 ± 2500 vs 7200 ± 800 copies per nanogram mRNA, P = .19). SGLT2 was not detectable in any myocardial sample.

4. Discussion

This is the first report on glucose-transporter–mediated functional effects of insulin in atrial myocardium. The results show that (1) insulin exerts a partially substrate-dependent

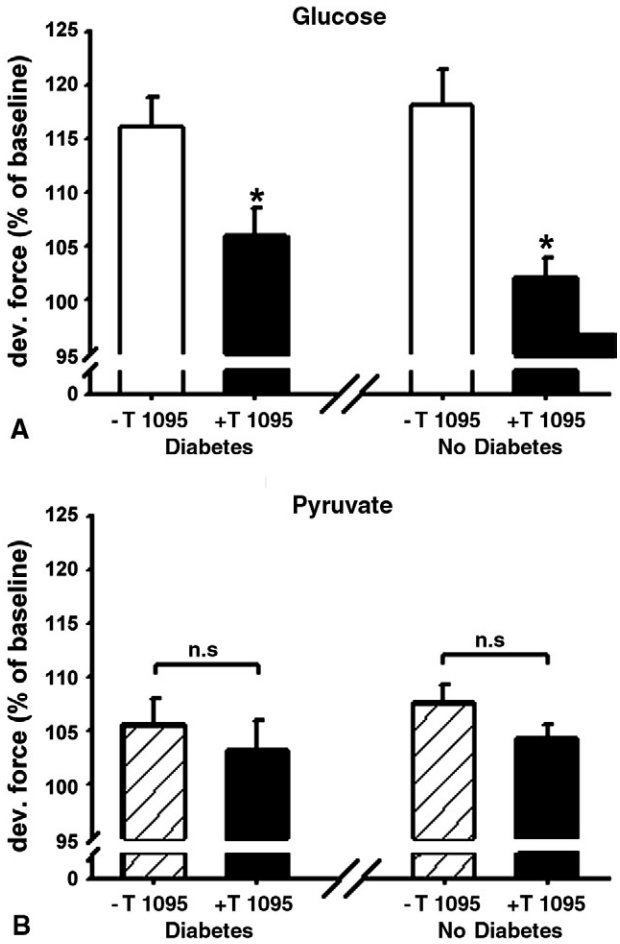

Fig. 4. A, Effect of insulin (0.02 μmol/L) in myocardial muscle strips with and without preincubation with SGLT1 blocker T1095A. Muscle strips from patients with diabetes (−T1095A, n = 10; +T1095, n = 16; left) or without diabetes (−T1095A, n = 5; +T1095A, n = 6; right). Energetic substrate in the solution is 11.2 mmol/L glucose. Control muscle strips without preincubation with T1095A originate from the same hearts. *P < .05 vs control. B, Effect of insulin (0.02 μmol/L) in myocardial muscle strips with and without preincubation with SGLT1 blocker T1095A. Muscle strips from patients with diabetes (−T1095A, n = 7; +T1095, n = 7; left) or without diabetes (−T1095A, n = 8; +T1095A, n = 10; right). Energetic substrate in the solution is 22.4 mmol/L pyruvate. Control muscle strips originate from the same hearts. *P < .05 vs control.

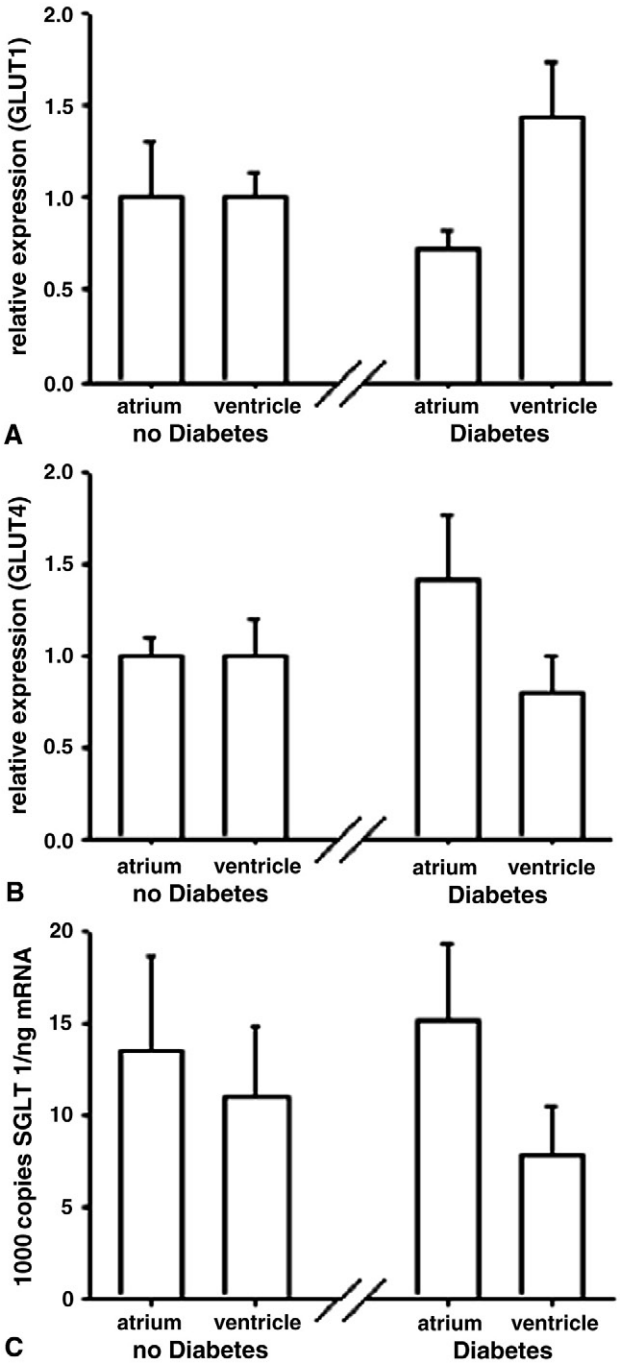

Fig. 5. A, GLUT1 mRNA expression in atrial and ventricular tissue samples of patients with diabetes (left; atrial, n = 13; ventricle, n = 13) and without diabetes (right; atrial, n = 14; ventricle, n = 15). Expression is plotted as relative expression compared with nondiabetic myocardium. B, GLUT4 mRNA expression in atrial and ventricular tissue samples of patients with diabetes (left; atrial, n = 13; ventricle, n = 13) and without diabetes (right; atrial, n = 14; ventricle, n = 15). Expression is plotted as relative expression compared with nondiabetic myocardium. C, SGLT1 mRNA expression in atrial and ventricular tissue samples of patients with diabetes (left; atrial, n = 13; ventricle, n = 13) and without diabetes (right; atrial, n = 14; ventricle, n = 15). Expression is plotted as 1000 copies per nanogram mRNA.

positive inotropic effect in human atrial myocardium, (2) the effect is mediated via PI-3-kinase and glucose transporters GLUT4 and SGLT1, (3) functional response is not altered in patients with diabetes, and (4) mRNA expression of glucose transporters involved is not altered in diabetes.

4.1. Inotropic/hemodynamic effects of insulin in the human cardiovascular system

Insulin exerts acute hemodynamic as well as long-term genomic effects in the cardiovascular system. In vitro, administration of insulin to multicellular human ventricular preparations results in an acute positive inotropic effect [13]. In vivo, acute application of insulin to human healthy volunteers or patients with diabetes increases left ventricular ejection fraction [10]. The inotropic effect in the in vitro model is partially Ca^{2+} dependent and in other parts glucose dependent and involves PI3-kinase and reverse-mode Na^{+}/Ca^{2+}-exchanger activation [13]. The underlying mechanisms for the hemodynamic effects in healthy volunteers remain unclear.

Preexistence of diabetes did not result in major changes of insulin-mediated inotropy in isolated human ventricular myocardium. The above-mentioned in vivo study, however, reports greater effects of insulin in nondiabetic patients. This could be due to differences in coronary blood flow in the 2 groups because an invasive study demonstrated vasodilatory actions of insulin only in nondiabetic patients, whereas glucose metabolism was not altered [19]. On the other hand, differences in myocardial contractility upon insulin administration might play a role; and these differences could be based on altered protein expression of signal transduction proteins involved in the inotropic effect.

4.2. Direct functional effects of glucose transporters in isolated human myocardium

We observed an acute increase in developed force of approximately 11% upon administration of insulin in atrial muscle preparations. This effect was sensitive to PI3-kinase inhibition as previously shown in isolated working rat heart and human ventricular myocardium [13,24]. Moreover, the presented data show for the first time a functional role of SGLT1 in myocardial tissue, as both glucose transporters (GLUT4 and SGLT1) contribute to the positive inotropic effect of insulin. Inhibition of either transporter significantly attenuated the increase in developed force, and only in one subgroup (muscle strips of diabetic patients using glucose as energetic substrate after preincubation with T-1095A) was a significant increase in developed force after insulin detectable. In this context, it has to be noted that inhibitory effects on inotropic responses are overestimated in atrial human myocardium because of an intrinsic rundown of the muscle preparations. Therefore, a remaining positive inotropic response to insulin is likely after inhibition of glucose transport, although it was only significant in one subgroup of our model. Inhibition of glucose transport also

significantly reduced contractile function in Langendorff preparations of GLUT4 knockout hearts [22]. In this model, impaired function could be restored after pyruvate administration, underlining the role of glucose transport in mediating contractility.

The reduced inotropic effect of insulin in pyruvate-containing solution is most likely due to the unspecific inhibition of glucose transporters due to the lacking substrate. However, basal developed force tended to be higher in the pyruvate group, which is in line with the described positive inotropic effect of pyruvate [25-27]. Because an increase in basal contractility lowers every additional positive inotropic stimulus if calculated as a percentage of basal force, this could also account for the differences.

The inotropic response to insulin was tested at 20 nmol/L in the present study, comparable to those concentrations used recently in ventricular human myocardium. Although this concentration is significantly lower than those used in previous studies in guinea pig and rat hearts [12], it is still considerably higher than postprandial plasma insulin levels in healthy human volunteers [28]. Although higher plasma insulin concentrations are likely to be reached using insulin glucose potassium infusions [29-31], the concentrations used in the presented study are supraphysiologic and supratherapeutic. Nevertheless, glucose-transport–mediated inotropic effects are a hitherto unknown feature of insulin action in human myocardium and might influence myocardial function in vivo.

4.3. No alterations of functional effects in diabetes

Glucose metabolism is severely altered in diabetes and involves multiple organs. Data on myocardial glucose metabolism are less detailed and controversial. Myocardial metabolism is shifted toward more fatty acids and less glucose (for review, see Taegtmeyer [32]). GLUT1 and GLUT4 as the major glucose transporters in rat myocardium are down-regulated in streptozotocin-induced diabetes in rats [33], and impaired transmembrane glucose transport is the main metabolic feature in myocardium of patients with diabetes. Defects in skeletal muscle glucose uptake can even be observed in healthy offspring of diabetic patients, underlining the genetic influence [34].

In our hands, functional effects of insulin were not different between preparations from diabetic patients and nondiabetic ones. The increase in developed force was 117.8% ± 2.5% in the former and 115.8% ± 1.9% in the latter group. In addition, signal transduction via PI3-kinase was not altered as can be seen from the experiments using the PI3-kinase inhibitor wortmannin, which reduced the positive inotropic effect to a comparable degree in both groups. Moreover, inhibition of GLUT4 translocation with latrunculin attenuated the positive inotropic effect of insulin to a similar degree in both groups; and blocking the SGLT1 with either phlorizin or T-1095A resulted in comparable reductions of the insulin effect. We have previously shown in human ventricular myocardium that inhibitory effects of wortmannin and T-1095A are additive and completely block the insulin-induced positive inotropic effect, whereas every single blocker only partially blocked the positive inotropic effect [35], indicating 2 distinct mechanisms.

In our hands, exchanging glucose with pyruvate as energetic substrate in the bathing solution resulted in reduced inotropic effects of insulin; but additional inhibition of glucose transporters did not result in any significant changes under this condition. This finding indicates that there are no or only minor unspecific functional effects of glucose transporter inhibition.

A reproducible finding in obese Zucker rats is a decrease in GLUT4 translocation at physiologic insulin levels [18,36] but unaltered transport at maximum insulin concentrations [18,37]. The insulin concentration we were using is still higher than maximum postprandial insulin serum concentrations. This could be an explanation for the similar results observed in the diabetic and the nondiabetic group.

4.4. No alterations of myocardial glucose transporter expression in diabetes

Besides functional effects, insulin mainly acts as a hormone regulating function and expression of a variety of proteins including glucose transporters. We therefore tested mRNA levels of SGLTs as well as the 2 main GLUTs, GLUT1 and GLUT4, in atrial and ventricular myocardium.

Metabolic genes are known to be regulated in heart failure with a shift further toward more glucose oxidation compared with nonfailing human myocardium [20] resembling the fetal gene pattern. However, this shift is accompanied by a reduction of the total amount of GLUT expression; and the GLUT4/GLUT1 ratio is shifted even further toward GLUT4 [20]. In contrast, diabetic cardiomyopathy is characterized by a strong reliance on fatty acid oxidation. In a rodent model with type 2 diabetes mellitus, GLUT4 protein content, glucose oxidation ,and glycolysis are significantly reduced [38]. Specific cardiac GLUT4 overexpression in this mouse model normalized myocardial glucose utilization. Moreover, the total amount of GLUT4 alterations in GLUT4 translocation has been described in models of diabetes.

Literature on myocardial SGLT1 is scarce. To our best knowledge, there is only one report on SGLT1 in myocardial tissue, by Zhou et al [21], reporting high RNA expression in samples of human hearts. Data on regulation of myocardial SGLT1 in diabetes do not exist; however, reports on the regulation in other organs describe an increase in intestine SGLT1 expression of patients with diabetes mellitus [39] and a more than 4-fold increase in renal SGLT1 expression in streptozotocin-induced diabetes mellitus in rats [40].

The present study did not find any significant changes between diabetic and nondiabetic patients with respect to glucose transporter expression. This is in line with data by Dutka et al [41] analyzing GLUT4 protein levels in myocardial biopsies of diabetic and nondiabetic patients.

They found unaltered protein levels despite a reduced glucose utilization and glucose uptake in myocardium of diabetic patients.

In conclusion, insulin-induced positive inotropic effect in human atrial myocardium is mediated by PI3-kinase and glucose transporters GLUT4 and SGLT1. SGLT1 expression and a functional role of this transporter in human atrium have been shown for the first time. Underlying diabetes mellitus did not change functional response or glucose transporter mRNA expression.

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