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Inverse regulation of glucose transporter Glut4 and G-protein G_s mRNA expression in cardiac myocytes from insulin resistant rats

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The present study examined the mRNA levels of glucose transporter Glut4 and G-protein G_s α-subunit in isolated ventricular myocytes from lean and genetically obese (*fa/fa*) Zucker rats and streptozotocin-diabetic rats. In obese animals the amount of transcripts coding for Glut4 increased to 122±6% of lean controls, whereas the mRNA coding for G_s α-subunit decreased by 42±12%. An unaltered level of G_s mRNA was observed in insulin deficient rats. When cardiomyocytes from normal rats were treated with insulin, the Glut4 transcript level increased by 48±5%, whereas the G_s mRNA level decreased by 55±8%. The findings suggest that insulin may act as a potential regulator of Glut4 and G_s mRNA expression in the cardiac cell.

Isolated cardiac myocyte; Diabetes; Insulin resistance; Glucose transporter; Guanine nucleotide-binding protein; Messenger ribonucleic acid

1. INTRODUCTION

Diabetes has been shown to be associated with substantial alterations of gene expression at both the protein and mRNA levels [1,2]. Several affected genes are thought to be of major importance for the pathogenesis of insulin resistance and these include the insulin responsive glucose transporter Glut4 [3–5] and the guanine nucleotide-binding proteins (G-proteins) [6,7]. Glut4 is exclusively expressed in fat and muscle and is responsible for the major part of insulin stimulated glucose uptake in these tissues [8,9]. On the other hand, G-proteins are known to couple many receptors to their effector systems and increasing evidence suggests that G-proteins are also involved in cellular signalling by insulin [10–12].

The relative contributions of altered Glut4 and G-protein expression to the insulin resistant state are presently unknown. Furthermore, tissue specific differences and differences between insulin-deficient diabetes and insulin resistance of obesity and Type II-diabetes have to be considered. Thus, in adipocytes [4] and skeletal muscle [3] of steptozotocin-diabetic rats the mRNA level of Glut4 was reported to be largely reduced. In contrast, no changes in the mRNA coding for G-protein α-subunits were observed in brain, muscle or kidney, whereas a significant reduction was detected in hepatocytes from insulin-deficient rats [13]. In skeletal muscle of obese Zucker rats [14] and *ob/ob* mice [15] the level of Glut4 protein was unchanged, whereas hepatocytes from obese Zucker rats exhibited a reduction in G_s α-subunit expression [16].

This laboratory has recently reported a large decrease in Glut4 mRNA expression in cardiomyocytes from streptozotocin-diabetic rats [12]. Furthermore, a functional association of Glut4 and a cholera-toxin-sensitive G-protein has been delineated [12]. In order to gain initial insights into the complex relationship between Glut4 and G-protein expression in diabetes, we have now performed the simultaneous determination of Glut4 and G_s α-subunit mRNA levels in ventricular myocytes from insulin resistant rats. The data suggest an inverse regulation of Glut4 and G_s mRNA expression by insulin.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

[γ-^{32}P]ATP (spec. radioact. 6000 Ci/mmol) and the G_s α-subunit oligonucleotide probe were purchased from New England Nuclear, Dreieich, Germany. Collagenase (EC 3.4.24.3) was from Serva, Heidelberg, Germany. The Glut4 oligonucleotide probe was synthesized by Promega, Madison, WI, USA. All other chemicals were analytical grade and supplied by Merck, Darmstadt, Germany.

2.2. *Animals*

Male Wistar rats fed ad libitum and weighing 280–320 g were used for the preparation of normal cardiomyocytes. Insulin-deficient diabetes was induced as described [17] by injecting citrate-buffered streptozotocin at a dosage of 60 mg/kg body wt., i.p. Genetically obese (*fa/fa*) male Zucker rats (12–16 weeks old) and their age-matched lean controls (*FA/?*) were bred in the animal laboratories of the Diabetes Research Institute. Blood samples were collected from the vena renalis immediately before starting perfusion of the hearts and taken for analysis of plasma glucose and insulin as previously described [18,19].

2.3. *Isolation of heart cells*

Calcium-tolerant myocytes were isolated by perfusion of the heart with collagenase as described in detail previously [20]. The final cell suspension was washed three times with HEPES buffer (composition NaCl 130 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM, HEPES 25 mM, glucose 5 mM, BSA 20 g/l, pH 7.4, equilibrated with O_2) and incubated at 37°C. $CaCl_2$ and $MgSO_4$ (final conc. 1 mM) were added and incubation was continued until further use.

2.4. *RNA isolation and Northern-blot analysis*

Total cellular RNA was isolated from ventricular myocytes by the guanidinium thiocyanate/phenol/chloroform method [21], separated by electrophoresis and transferred to nylon membranes as described [12]. Densitometry of ethidium bromide-stained 18 S rRNA was used to normalize the amount of RNA loaded per lane. Blots were hybridized to oligonucleotide probes which were 5'-end-labelled to a specific radioactivity of $(2\text{–}6)\times10^8$ dpm/μg by using [^{32}P]ATP and T4 kinase. After washing under low-(G_s) and high-stringency (Glut4) conditions, the blots were exposed to Kodak X-Omat AR film at −70°C by using intensifying screens. The autoradiographs were quantified by laser-scanning densitometry to determine the relative amounts of Glut4 and G_s mRNA. Multiple scans were made of each band and the results averaged. Samples from control and diabetic tissues were always run on the same blots, since the present method is valid only when comparing different samples analyzed in the same blot. Significance of reported differences was evaluated by using *t* statistics for paired and unpaired data.

3. RESULTS

All experiments were performed on ventricular cardiomyocytes isolated from normal Wistar rats, from low-dose streptozotocin-diabetic rats (blood glucose 30 ± 1 mM versus 6 ± 0.2 mM in controls; plasma insulin 7.0 ± 0.9 μU/ml versus 21.7 ± 3.5 μU/ml in controls) and from lean and obese Zucker rats (normoglycemic; plasma insulin 525 ± 198 μU/ml versus 27 ± 5 μU/ml in lean animals). As described in earlier reports from this laboratory [17,18], myocytes from diabetic animals were found to have an unaltered morphology, viability (80–90%) and ATP-content. The different groups of animals exhibited an unaltered yield of total RNA, which varied between 40–60 μg/10^6 cells.

The effect of obesity on glucose transporter expression was examined by probing Northern blots of total RNA from lean and obese Zucker rats with a 24mer oligonucleotide specific to Glut4 [12]. As shown in Fig. 1, a major transcript of 2.7 kb for both lean and obese rats was identified. Densitometric analysis of autoradiograms revealed a slight (122 ± 6% of control) but significant increase in Glut4 mRNA concentration. When the same analysis was performed using an oligonucleotide probe against the G_s α-subunit mRNA (constructed against position 580–618 [22]), one single transcript of 1.9 kb was observed which was reduced by 42 ± 12% in myocytes from obese Zucker rats (Fig. 2, left panel). In contrast, an unaltered G_s α-subunit mRNA level has been quantified in cardiomyocytes from insulin deficient rats (Fig. 2, right panel). This is completely different from the large decrease in Glut4 expression in heart myocytes from these animals [12].

Inverse regulation of Glut4 and G_s mRNA expression could be related to the high plasma insulin levels of

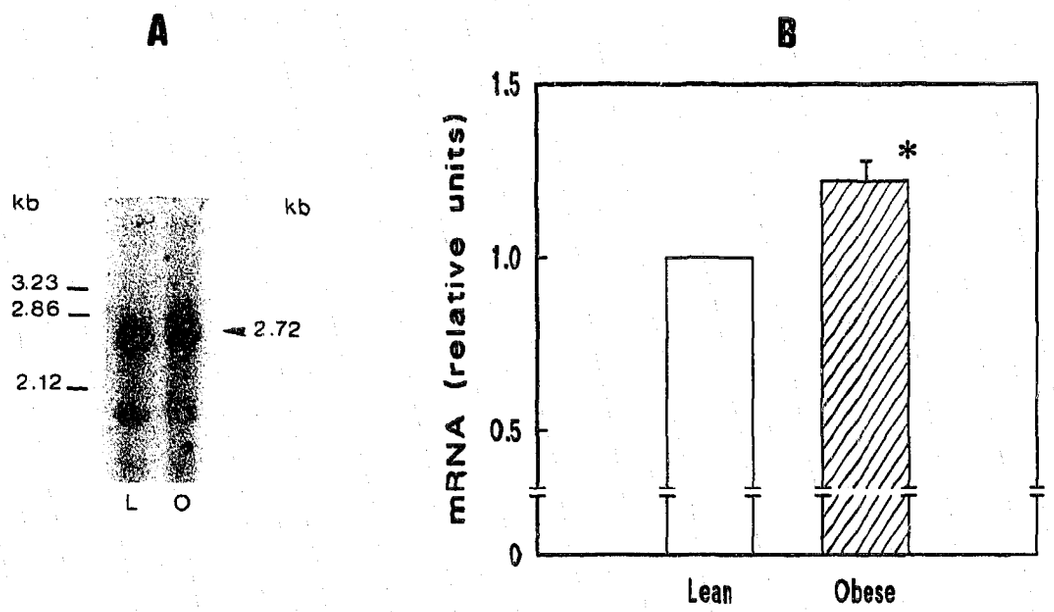

Fig. 1. Northern-blot analysis of Glut4 mRNA expressed in cardiac myocytes from lean (L) and obese (O) Zucker rats. (A) Total RNA was electrophoresed (10 μg/lane), transferred to a nylon membrane and hybridized with a ^{32}P-labelled oligonucleotide probe complementary to Glut4. Autoradiograms were exposed for 2 days. The position of RNA size markers is indicated on the left. (B) mRNA was quantified from autoradiograms by laser-scanning densitometry relative to the amounts in controls, which was assigned a value of 1.0. Data are means ± SE (n = 3). *Significantly different from lean control (P = 0.034)

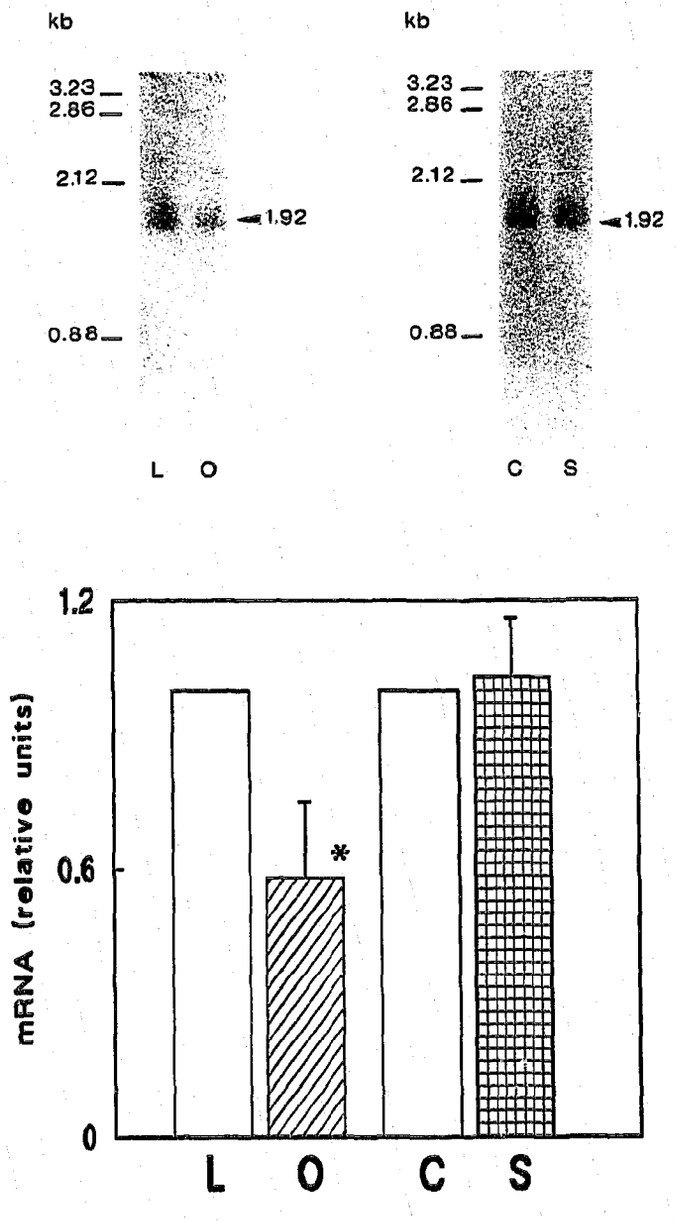

Fig. 2. Detection of mRNA encoding the G_s α-subunit in cardiac myocytes from diabetic rats. L, lean Zucker rat; O, obese Zucker rat; C, control; S, streptozotocin-diabetes. Upper panel: 5 μg of total RNA per lane. Blots were hybridized with a radiolabelled oligonucleotide probe complementary to G_s α-subunit. Exposure time was 6 days. Lower panel: Quantification of mRNA was performed as outlined in Fig. 1. Data are means ± SE ($n=4$). *Significantly different from lean control ($P=0.048$)

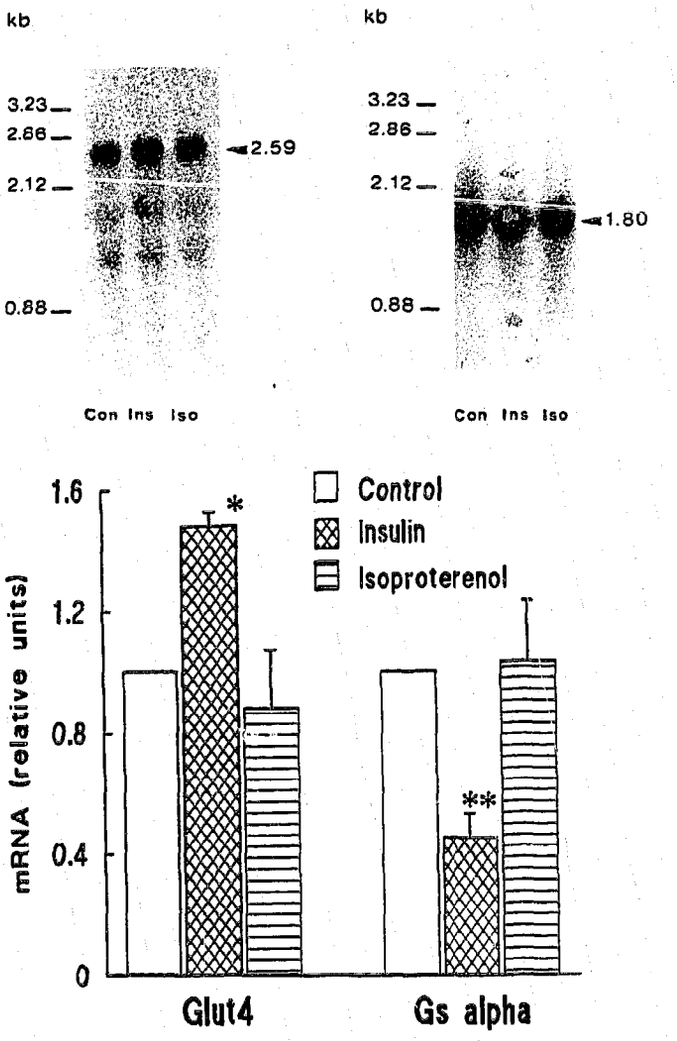

Fig. 3. Effect of insulin and isoproterenol on the levels of Glut4 (left panel) and G_s α-subunit (right panel) mRNA in isolated cardiac myocytes. Cells were incubated for 4 h at 37°C in the absence (control) or presence of insulin (10^{-6} M) or isoproterenol (10^{-4} M). Each lane contains 10 μg total RNA. Hybridization and quantification of mRNA were performed as outlined in Figs. 1 and 2. Data are means ± SE ($n=3$-5). Significantly different from control *($P=0.005$), **($P=0.002$).

obese Zucker rats. Therefore, cardiac myocytes from normal Wistar rats were incubated with the hormone for 4 hours and processed for Northern-blot analysis of total RNA. Under these conditions the mRNA level of Glut4 was increased by 48 ± 5%, whereas the G_s α-subunit mRNA level decreased by 55 ± 8% (Fig. 3). Isoproterenol had no effect on the transcript concentrations of both Glut4 and G_s α-subunit.

4. DISCUSSION

Reduced Glut4 expression most probably contributes to the pathogenesis of insulin resistance in insulin-deficient diabetes and has been observed in all target tissues [3-5,12]. A more complex situation and an apparent difference between fat and muscle may be found in other states of insulin resistance like obesity and Type II-diabetes. Thus, a large decrease in Glut4 gene expression detected in adipocytes from patients with obesity or NIDDM [23] contrasts with an unaltered Glut4 expression in skeletal muscle of such patients [24]. Our results now show that Glut4 mRNA expression even slightly increases in cardiac muscle from obese Zucker rats. This

finding agrees in principle with recent reports of an unaltered Glut4 expression in skeletal muscle of obese mice [15,25] and Zucker rats [14], and supports the notion that Glut4 expression is regulated in a tissue-specific way [25,26].

This laboratory has recently reported the existence of both receptor and postreceptor-defects when characterizing the resistance of insulin stimulated glucose uptake in cardiomyocytes from obese Zucker rats [18]. Basal transport and maximal responsiveness were largely reduced in agreement with observations in the perfused heart [27]. The present study shows that this defect is accompanied by an increase in Glut4 mRNA expression and therefore suggests that the defect in glucose transport may be related to changes in intrinsic activity or translocation of Glut4 or to altered transmembrane signalling by insulin.

In contrast to Glut4, a substantial reduction in the mRNA coding for G_s α-subunit was observed in cardiomyocytes from obese rats, whereas an unaltered level was determined in streptozotocin-diabetic rats. The latter finding agrees well with a recent report by Griffiths et al. [13], who described an unaltered expression of G_s α-subunit mRNA in the streptozotocin-diabetic rat heart. The functional implications of reduced G_s mRNA expression in obesity remain to be elucidated. It has been proposed that G_s may interact with the glucose transporter [28] and it may be speculated that reduced expression of G_s is related to the defect in cardiac glucose transport. This issue needs further investigation.

The inverse regulation of Glut4 and G_s mRNA expression observed in obese Zucker rats could be completely mimicked by incubating cardiomyocytes from normal rats with insulin for 4 h, suggesting that the high plasma insulin levels might be responsible for altered gene expression. Indeed, insulin has been implicated in Glut4 gene expression [29], although tissue specific differences seem to be involved. Thus, in L6 muscle cells [29] Glut4 mRNA expression was decreased by prolonged administration of insulin. On the other hand, in hyperinsulinemic rats [30] Glut4 mRNA increased in adipose tissue but decreased in skeletal muscle. In contrast to Glut4, hormone responsive elements have already been identified in the 5′ non-coding region of G-protein α-subunit genes [31] and regulation of G-protein expression by steroids [32] and thyroid hormones [33] has been reported. To our knowledge, the present study is the first report of a direct effect of insulin on G-protein mRNA expression. Thus, in addition to signalling via G-proteins [12], insulin may regulate the level of these signal transducing elements.

Taken together, reciprocal changes of Glut4 and G_s α-subunit mRNA levels have been observed in cardiomyocytes from obese rats. The data suggest that insulin may act as a potential regulator of Glut4 and G_s mRNA expression in the cardiac cell.

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