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Human Myocardial mRNA-Expression of Insulin-Dependent Transmembrane Glucose Transporter is Increased in Human IDDM and Decreased in NIDDM

S. Gasser¹, L. Cichocki¹, S. Barth¹, W. Klein(†)¹, I. Friehs², B. Rigler³, H. Mächler³, R. Gasser¹

Transmembrane glucose transport and thus cellular high energy metabolism of the cardiovascular system depend largely on the insulin responsive GLUT4-isoform of the transmembrane glucose transport molecule. Several authors have shown that, in animals, myocardial GLUT4-mRNA expression is decreased in experimental diabetes. There are no data on humans as yet.

Here, we investigate probes of right atrial auricle from diabetic and non diabetic patients subjected to cardiac surgery which were snap frozen in liquid nitrogen. Semiquantitative PCR has been used in order to quantify GLUT4-mRNA using G3PDH as a house keeping gene.

Our results represent the first measurements of GLUT4-mRNA in human myocardial tissue. Seven patients had NIDDM (determined by OGT, HbA1C and insulin secretion), 7 had IDDM and 7 served as controls. Relative GLUT4-expression amounted to 97.0 ± 10.4 ($\pm$ SEM) in the control group, 133.3 ± 15.2 in the IDDM-group and 49.1 ± 6.2 ($\pm$ SEM) in the NIDDM-group. From these data we deduce that DM is initially associated with a decreased GLUT4-expression, the latter then being upregulated by external application of insulin. *J Clin Basic Cardiol* 2004; 7: 8–10.

Key words: glucose transport, gene expression, real time PCR, hypertension

In mammals, the transport of glucose across cell membranes occurs by facilitated diffusion. Several cDNAs encoding structurally related proteins with the properties of facilitative glucose transporters have been isolated and characterized (GLUT). These molecules regulate transmembrane glucose transport in various tissues. GLUT4 appears to be of special interest for several reasons, in particular, because it is the only GLUT which is directly regulated/stimulated by insulin. It is found in various tissues like cardiac and skeletal muscle, as well as adipose tissue [1]. The isolation and characterisation of a monoclonal antibody that specifically recognised this “muscle-fat isoform” GLUT4 has revealed that it was a unique isoform, different from the glucose transporters present in erythrocytes, brain, kidney, jejunum and liver. It shows between 50 and 70 % cDNA identity with GLUT1–3. Insulin causes a rapid and reversible increase in glucose transport activity via GLUT4 in cardiac and skeletal muscle [2].

While different types of muscle fibres contain different levels of GLUT4-proteins and gene expression as well as different insulin sensitivities, the nutritional state and contractile activity appear to regulate GLUT4-gene expression. Fasting, for example, results in a two to threefold increase in GLUT4-protein and gene expression in mixed soleus and gastrocnemius muscle preparations [3]. Exercise training also increases GLUT4-protein levels in rat skeletal muscle [4], whereas there are conflicting results concerning patients with NIDDM: In skeletal muscle, Handberg and co-workers found no significant difference in the levels of GLUT4-mRNA and protein in biopsies from patients with and without NIDDM [5], whereas Dohm et al found a significantly decreased expression of GLUT4 in skeletal muscle from insulin resistant patients [6]. In rat cardiac tissue of streptozotocin-diabetic rats, Eckel and Reinauer have shown that GLUT4-mRNA is decreased [7]. Interestingly, in human heart, preliminary data of our own group show that, in NIDDM-patients, GLUT4-mRNA expression is down-

regulated [8], whereas it is upregulated in IDDM [9]. Here we present data from patients with IDDM and NIDDM compared with controls. We find that myocardial mRNA expression of insulin-dependent transmembrane glucose transporter is increased in human insulin-dependent diabetes mellitus and decreased in non-insulin-dependent diabetes mellitus compared to control subjects.

Methods

Myocardial tissue probes derive from the right auricle of patients undergoing cardiac surgery. A small part of the right auricle is removed when the heart is put on extracorporeal circulation and is normally wasted. The muscle piece (60–200 mg) will then be snap frozen in liquid NO and stored at -70°C until homogenisation. Total RNA was isolated using guanidium thiocyanate, phenol-chloroform extraction and alcohol precipitation. Total RNA was hybridised with 32P-labelled human GLUT4-cDNA and re-hybridised with a human G3PDH-cDNA probe to correct for equal amounts of RNA. Quantification was performed by a laser scanner and is expressed in optical densities.

Results

Our results represent one of the first measurements of GLUT4-mRNA in human myocardial tissue. Seven patients had NIDDM (determined by OGT, HbA1C, and insulin secretion), 7 had IDDM and 7 served as controls. GLUT4-mRNA expression was 97.0 ± 10.4 ($\pm$ SEM) in the control-group, 133.3 ± 15.2 in the IDDM-group and 49.1 ± 6.2 in the NIDDM-group.

One can see that, in patients with IDDM, relative optical densities reflecting the amount of GLUT4-mRNA are higher, as expressed in examples in Figure 1: 130.4 and 131.8 RODs calculated as the integral of total area corrected the

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absolute density of the house-keeping gene G3PDH. 32 P-labelled human GLUT4-cDNA had been used for hybridisation. While control values tended to be around 100 RODs (97.0 ± 10.4), NIDDM-patients showed RODs of around half of that, when corrected for the house-keeping gene.

The fact that insulin treated diabetic patients exhibit a down-regulated GLUT4-expression is surprising. However, the facilitated glucose entry into the cell in the presence of higher concentrations of serum insulin may cause the observed down-regulation of transmembrane insuline dependent glucose transport molecules.

Discussion

The interrelation between disturbances in glucose-metabolism, hypertension and myocardial ischemic disease has been known for a long time and thus has been the subject of investigation in a multitude of trials, publications and experimental studies [c.f. 10–19]. Insulin resistance and reactive hyperinsulinemia occur not only with obesity, impaired glucose tolerance or non-insulin-dependent (type 2) diabetes mellitus, but also in many non-obese [20], non-diabetic patients with essential hypertension and seem to be largely responsible for the development of hypertension. The common co-existence of genetic predisposition for hypertension with insulin resistance helps to explain the frequent, although temporally often dissociated, occurrence of hypertension together with dyslipidemia, obesity and type 2-diabetes in a given cohort. In the pathogenesis of metabolic syndrome, inappropriate vasoconstriction, structural changes of the cardiovascular system [21–23] as to its stiffness, but also unfavourable distribution of liquid between the compartments play a key role. While the complete cascade of interactions between glucose-metabolism, KHK, obesity and hypertension has not been elucidated completely as yet, trans-membrane glucose transport is certainly crucial in this setting [1–9].

GLUT4, the insulin-dependent transmembrane glucose facilitative transport molecule, plays a decisive role in insulin-dependent cardiac glucose metabolism, apparently also for myocardial [24] and vascular stiffness [19] as well as in the context of osmolarity, compartmental water distribution and homeostasis [1]. As early as 1995, comparative studies using nuclear magnetic spectroscopy in heart of normotensive (WKY) and spontaneously hypertensive rats (SRH) have looked at glucose uptake during insulin stimulation as well as

mRNA-expression of GLUT1 and GLUT4 [25]: in hypertensive rats, expression of GLUT4-mRNA as well as the amount of protein in the membrane had been decreased and cardiac hypertrophy increased by 59 %. Similar results have been found in the afferent vessel of the renal glomeruli in experimental, streptozotocin-induced diabetes mellitus. In diabetic animals, GLUT4 as well as polypeptide expression and thus glucose uptake had been reduced. In this context, it has been speculated that the resulting decrease of GLUT4 could modulate renal blood flow and, in turn, lead to hypertension. We then concluded that defective GLUT4-expression may also occur in human myocardium of diabetics [8–10] as well as hypertensives [26]. Disturbed transmembrane glucose transport may also significantly contribute to the development of severe coronary heart disease [27] and diabetic cardiomyopathy [28]. In the context of hypertension, very few authors have looked at evidence for myocardial and vascular GLUT4-involvement in the development of hypertension in animals [29] and still no reports can be found on GLUT4 in human myocardium. Despite the scant experimental direct evidence, Ikegami et al have already postulated the GLUT4-gene as one of the target genes in essential hypertension when accompanied with insulin resistance [30].

In the present project we have intensified our investigational efforts focussed on the unknown role played by GLUT4 in the development of different forms of diabetes in the context of the above considerations.

GLUT4 and Myocardial Ischemia

Glucose and high energy metabolism play a pivotal role in the development of numerous salient characteristics of myocardial ischemia, such as the gating properties of specific ion-channels, intracellular ion-homeostasis, electrical phenomena, contractility and other phenomena [31–33]. Many of these aspects of myocardial ischemia are linked in one or the other way to transmembrane glucose transport, intracellular glucose metabolism and, in fact, to GLUT4 [34–36]. Myocardial ischemia increases glucose uptake through translocation of GLUT1 and GLUT4 from an intracellular compartment to sarcolemma. This appears to be a beneficial effect during ischemia and possibly recovery. Insulin and ischemia have additive effects to increase *in vivo* glucose utilisation and augment glucose transporter translocation [37]. Delivery of glucose to the glycolytic pathway appears to be a major controlling site of glycolysis in low-flow ischemia. Downstream regulation is then distributed along the pathway with no one site exerting greater inhibition than reduced glucose delivery [38]. While many experimental studies suggest that an increase in glucose uptake and metabolism by the ischemic myocardium helps to protect myocardial cells from irreversible injury [39], little or nothing is known in this context about human cardiac transmembrane glucose transport, GLUT4-expression and the interrelation between the latter and diabetes during ischemia.

The weakness of the study is twofold: on the one hand, the patient cohorts are relatively small and hence difficult to match concerning co-medications, age and co-morbidities. However, it can be argued that the differences between the groups are statistically relevant, and that the data are certainly valid to inspire larger investigations. Furthermore, it has to be argued that, in the present setting, it is difficult to retrieve sufficient human material in order to aim at larger cohorts. The second weakness targets the semiquantitative method of measurement. RT-PCR would certainly constitute a more

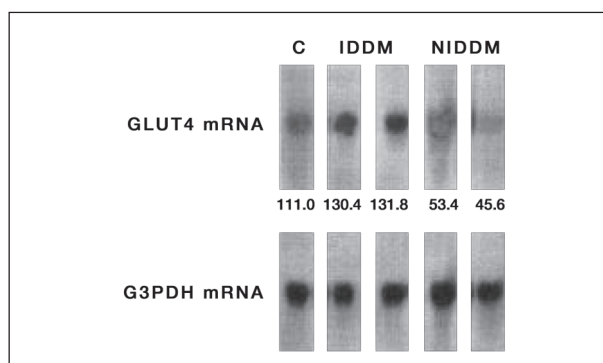


Figure 1. Northern Blot of total RNA with 32 P-labeled human GLUT4-cDNA of patients with NIDDM, IDDM and controls. Rehybridization with a human G3PDH-cDNA probe to correct for equal amounts of RNA. Quantification was performed by laser scanner and is expressed in optical densities.

elegant methodological approach. Hence, inspired by the above measurements, we began to establish real time PCR for the assessment of GLUT4-mRNA under various conditions. Further investigations on the subject are certainly needed in order to elucidate the role played by GLUT4 in the development of diabetes in humans.

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References

- Pessin JE, Bell GI. Mammalian facilitative glucose transporter family: structure and molecular regulation. *Ann Rev Physiol* 1992; 54: 911–30.
- James DE, Brown R, Navarro J, Pilch PF. Insulin-regulatable tissues express a unique insulin-sensitive glucose transport protein. *Nature* 1988; 333: 183–5.
- Berger J, Biswas C, Vicario PP, et al. Decreased expression of the insulin-responsive glucose transporter I diabetes and fasting. *Nature* 1989; 340: 70–2.
- Friedman JE, Sherman WM, Reed MJ et al. Exercise training increases glucose transporter protein GLUT4 of obese Zucker (fa/fa) rats. *FEBS Lett* 1990; 268: 13–6.
- Handberg A, Vaag A, Damsø P, et al. Expression of insulin regulatable glucose transporters in skeletal muscle from type 2 diabetic patients. *Diabetologia* 1990; 33: 625–7.
- Dohm GL, Pelton CW, Friedmann JE, et al. Decreased expression of GLUT4 in skeletal muscle from insulin resistant patients. *Am J Physiol* 1991; 260: E459–E463.
- Eckel J, Reinauer H. Insulin action on glucose transport in cardiac myocytes: signalling pathways and diabetes induced alterations. *Biochem Soc Trans* 1990; 18: 1125–7.
- Barth S, Klein W, Friehs I, Rigler B, Zechner R, Gasser R. GLUT4 mRNA expression in human myocardium is altered in patients with non-insulin-dependent diabetes mellitus. *Eur Heart J* 1996; 17 (Suppl): 475.
- Brussee H, Barth S, Klein W, Friehs I, Rigler B, Gasser R. Myocardial mRNA expression of insulin-dependent transmembrane glucose transporter is increased in human insulin-dependent diabetes mellitus and decreased in non-insulin-dependent diabetes mellitus. *Eur Heart J* 1999; 20 (Suppl): 207.
- Kickenweiz E, Barth S, Friehs I, Rigler B, Zechner R, Köppel H, Klein W, Gasser R. Molekulare und genetische Ursachen der Hypertonie. *Jatros Kardiologie* 1999; 4: 22–7.
- Kaplan NM. Clinical Hypertension. A monograph. Williams & Wilkins, Baltimore, Sydney, Tokyo, 2002.
- Brown MJ. The causes of essential hypertension. *Br J Clin Pharmacol* 1996; 42: 21–7.
- Barth S, Kickenweiz E, Friehs I, Rigler B, Zechner R, Köppel H, Gasser R. Hypertonie – Symptom oder Krankheit? *J Hyperten* 1999; 4: 8–15.
- Riva-Rocci S. *Gaz med di Torino* 1886.
- O'Shaughnessy M, Kotchen A. Epidemiologic, physiologic and clinical implications of hypertension and insulin resistance. *Curr Opin Cardiol* 1993; 8: 757–64.
- Kristiansson K, Sigfusson N, Sigvaldson H, Thorgeirsson G. Glucose tolerance and blood pressure in a population-based cohort study of males and females: the Reykjavik Study. *J Hyperten* 1995; 13: 581–6.
- Weidmann P, Böhlen L, de Courten M. Insulin resistance and hyperinsulinemia in hypertension. *J Hyperten* 1995; 13: 565–72.
- Gasser R. The interdependence of hypertension, calcium overload and coronary spasm in the development of myocardial infarction. *Angiology* 1988; 39: 761–72.
- Atkins KB, Johns D, Watts S, Clinton Webb R, Brosius FC. Decreased vascular glucose transporter expression and glucose uptake in DOCA-salt hypertension. *J Hyperten* 2001; 19: 1581–7.
- Marengo C, Macchia G, Putigna G, Accomazzo L, Pagano A, Cassader M, Disegni A and Pagano G. Hyperinsulinemia and metabolic variables in normal weight essential hypertensive patients. *Nutr Metab Cardiovasc Dis* 1995; 5: 211–6.
- Nyses L, Peltzer T. Molekulare Adaptation des Herzens an Hypertonie. *Z Kardiol* 1995; 84 (Suppl 4): 71–6.
- Ritter O, Neyses L. The molecular basis of myocardial hypertrophy and heart failure. *Trends Mol Med* 2003; 9: 313–21.
- Katzer E, Barth S, Köppel H, Gasser R, Klein W, Friehs I, Rigler B. EGR-1 messenger RNA expression in right atrial auricle human myocardium is upregulated in hypertensive patients with myocardial hypertrophy. *Proceedings World Congress of Cardiology 1998 – Rio de Janeiro, Selected Papers. Monduzzi Editore, Bologna*; 687–92.
- Kaczmarczyk SJ, Andrikopoulos S, Favaloro J, Domenighetti, et al. Threshold effects of glucose transporter-4 (GLUT4) deficiency on cardiac glucose uptake and development of hypertrophy. *J Mol Endocrinol* 2003; 31: 449–59.
- Paternostro G, Clarke K, Heath J, Seymour AM, Radd GK. Decreased GLUT4 mRNA content and insulin-sensitive deoxyglucose uptake show insulin resistance in the hypertensive rat heart. *Cardiovas Res* 1995; 30: 205–11.
- Klein W, Gasser R, Barth S, Friehs I, Rigler B, Zechner R. GLUT4 mRNA expression in human myocardium is reduced in hypertension. 4th Alpe Adria Cardiology Meeting – Grado, 1996: Abstract book.
- Schwaiger M, Sun D, Deed GM, Nguyen N, Haas F, et al. Expression of myocardial glucose transporter (GLUT) mRNAs with advanced coronary heart disease (CAD). *Circulation* 1994; 90 (Suppl I): 113.
- Garvey WT, Hardin D, Juhaszova M, Dominguez JH. Effects of diabetes on myocardial glucose transport system in rats: implications for diabetic cardiomyopathy. *Am J Physiol* 1993; 264: H837–H844.
- Campbell IW, Dominiczak AF, Livingstone C, Gould GW. Analysis of the glucose transporter complement of metabolically important tissues from the Milan hypertensive rat. *Biochem Biophys Res Commun* 1995; 211: 780–91.
- Ikegami H, Yamato E, Fujisawa T, Hamada Y, Fujioka Y, Rakugi H, Higaki J, et al. Analysis of candidate genes for insulin resistance in essential hypertension. *Hyperten Res* 1996; 19: S31–4.
- Gasser R, Klein W. K_{ATP} -channels, action potential shortening and contractile failure in early myocardial ischemia, a review (invited paper). *Cardiovasc Drugs Ther* 1994; 8: 813–22.
- Gasser R, Vaughan-Jones RD. Mechanism of potassium efflux and action potential shortening during ischemia in isolated mammalian cardiac muscle. *J Physiol (London)* 1990; 431: 713–41.
- Tong H, Chen W, London RE, Murphy E, Steenbergen C. Preconditioning enhanced glucose uptake is mediated by p38 MAP kinase not by phosphatidylinositol 3-kinase. *J Biol Chem* 2000; 275: 11981–6.
- Tian R, Abel ED. Responses of GLUT4 deficient hearts to ischemia underscore importance of glycolysis. *Circulation* 2001; 103: 2961–6.
- Egert S, Nguyen N, Schwaiger M. Myocardial glucose transporter GLUT1: translocation induced by insulin and ischemia. *J Mol Cell Cardiol* 1999; 31: 1337–44.
- Egert S, Nguyen N, Schwaiger M. Contribution of alpha-adrenergic and beta-adrenergic stimulation to ischemia-induced glucose transporter (GLUT) and GLUT1 translocation in the isolated perfused rat heart. *Circ Res* 1999; 84: 1407–15.
- Russell RR, YIN R, Caplan MJ, et al. Additive effects of hyperinsulinemia and ischemia on myocardial GLUT1 and GLUT4 translocation in vivo. *Circulation* 1998; 98: 2180–6.
- King LM, Opie LH. Glucose delivery is a major determinant of glucose utilization in the ischemic myocardium with a residual coronary flow. *Cardiovasc Res* 1998; 39: 381–92.
- Brosius FC, Nguyen N, Egert S, Lin Z, et al. Increased sarcolemmal glucose transporter abundance in myocardial ischemia. *Am J Cardiol* 1997; 80: 77A–84A.

Further Reading

- Gasser R, Schafhalter I, Wolff P, et al. Experimental models and definition of myocardial ischemia. *Internat J Angiol* 1994; 3: 154–6.
- Razeghi P, et al. Metabolic gene expression in fatal and failing human heart. *Circulation* 2001; 104: 2923–31.
- Depre C, et al. Unloaded heart in vivo replicates fetal gene expression of cardiac hypertrophy. *Nature Medicine* 1998; 4: 1269–75.
- Gasser R, Köppel H, Horn S, Wallner M, et al. Modulation of glycolysis affects vascular response to sulfonylureas: a study on isolated bovine coronary artery strips. *Exp Clin Cardiol* 1999; 4: 59–63.

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