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Shift from Adult to Fetal Metabolic Phenotype During Prolonged Experimental Myocardial Ischemia: A Study on the Effect of Beta Blockers upon Gene Expression of Transmembrane Glucose Transporters

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The fetal myocardial phenotype predominantly uses glucose for its metabolism, whereas the adult individual mainly metabolises fatty acids. During special conditions, like hypoxia and exercise, the adult phenotype of myocardial metabolism converts to the fetal one, again preferably using glucose as a substrate. It has been shown that a preferentially glucose-oriented cardiac metabolism is beneficial in myocardial ischemia.

Our own microarray experiments confirm those data. Here we find that gene expression of biological processes which are associated with glucose metabolism are up-regulated during hypoxia, whereas those associated with fatty acid and amino-acid metabolism are down-regulated. Testing the effects of beta blockers (atenolol and nebivolol) we find a similar shift in well-oxygenized preparations, suggesting that the cardioprotective action of beta blockers is brought about by a shift from the adult to the fetal phenotype of metabolism.

Myocardial ischemia thus increases glucose uptake through translocation of GLUT1 and GLUT4 from an intracellular compartment to the sarcolemma. This appears to be beneficial during ischemia and possibly recovery. Here we find that there is no significant regulation with and without the influence of beta blockers during myocardial ischemia – there is, however, a significant difference between the expression of GLUT1 in well-oxygenized preparations with (0.087 ± 0.02) and without nebivolol (0.62 ± 0.02; ± SEM; $p \leq 0.05$). Similarly, atenolol led to an increase of GLUT1 expression in well-oxygenated preparations compared to controls: 1.18 ± 0.08 and 0.62 ± 0.02, respectively (± SEM; $p < 0.05$). While there is no significant regulation with and without the influence of beta blockers during myocardial ischemia, there is, however, a significant difference between the expression of GLUT4 in well-oxygenized preparations with (0.52 ± 0.01) and without nebivolol (0.29 ± 0.02; ± SEM; $p \leq 0.05$). Similarly, atenolol led to an increase of GLUT4 expression in well-oxygenated preparations compared to controls: 0.92 ± 0.10 and 0.29 ± 0.02, respectively (± SEM; $p < 0.05$).

These results mirror the increased demand of glucose as a substrate in the presence of beta blockers.

Shifting myocardial metabolism to the fetal phenotype has become a new target for anti-anginal treatment in the aging heart, either by augmentation of glucose metabolism or by inhibiting fatty acid metabolism. The latter has been successfully targeted by drugs like trimetazidine and ranolazine. In summary, it has been shown for the first time that some of the anti-anginal effects of beta blockers may possibly be conveyed by their action on GLUT1/4 expression in myocardial cells by facilitating glucose metabolism and in turn causing a shift to the fetal phenotype of metabolism in the adult human heart. ***J Clin Basic Cardiol 2009; 12 (online): 11–7.***

Key words: *metabolism, myocardial ischemia*

Myocardial ischemia constitutes a serious condition which has been under investigation for decades. Much of its pathophysiology is well understood, however, the understanding is limited by the complexity of the phenomenon. Severity and appearance of ischemia are time- and substrate-dependent and there is a difference between total, partial, and intermittent ischemia in outcome [1–3]. Newer techniques like microarray gene expression analysis have shed new light upon the molecular processes involved. Hundreds of genes are regulated during ischemia and reperfusion. Some of those indicate repair, others injury [4].

During and after myocardial ischemia, beta blockers support the preservation of myocardial integrity [5]. Since nebivolol shows numerous pharmacological properties which may entail anti-ischemic protection, we analysed the molecular signature by quantitative assessment of expression of approximately 27,000 genes in the human atrial myocardium under ischemic conditions in the presence and absence of nebivolol [6]. Preliminary measurements by our group have thus indicated numerous pathways and biological processes differentially expressed when experimental ischemia is performed in the presence of nebivolol (see below) [6]. While it has been shown by a number of authors that, in experimental/clinical myocardial ischemia, gene expression is altered, gene expression profiling in ischemia has not been looked at in human myocardial tissue in depth so far. Hence, it has remained unknown whether or not ischemia can be related to specifically altered patterns of gene expression. The latter, as a particular molecular signature, could reveal a new understanding of cardio-protective mechanisms. Using this technique, relevant cardio-protective properties of anti-ischemic drugs could be more easily accessed. In order to gain more understanding of the relevant molecular pathways and biochemical processes, the effect of beta blockers has been looked at.

In the present work we studied the effects of ischemia on mRNA expression in experimentally ischemic human myocardial tissue. While many experimental studies suggest that changes in gene expression within the ischemic myocardium help to protect myocardial cells from irreversible injury, little has been known in this context about ischemic gene-expression in human cardiac tissue and the interrelation between the latter and cardio-protective agents [5–7].

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Glucose utilization in the myocardium is controlled by many factors including cardiac work, ischemia, hypoxia, and insulin [8]. Diabetic myocardium and hypertrophied myocardium, 2 states of relative insensitivity to insulin, appear to have an impaired ability to increase glucose uptake in response to either insulin or ischemia [9–13]. In mammalian cells, glucose is not freely permeable across the lipid bilayer; it enters the cells by facilitated diffusion. Specific membrane proteins that passively transport glucose down a concentration gradient achieve this process. In cardiac myocytes, several types of glucose transporters have been described; GLUT1 and GLUT4 are mainly responsible for most of the uptake of glucose under basal conditions and in response to insulin stimulation, respectively (see also [14]). Impaired glucose uptake due to an intrinsic defect in insulin-mediated glucose uptake may result in decreased tolerance of the myocardium to ischemia and impaired recovery of post-ischemic contractile function [10]. The GLUT1 transporter is widely expressed and is the major mediator of glucose uptake in tissues such as the brain, the blood-brain barrier, erythrocytes, and endothelial cells [14, 15].

GLUT4 and GLUT1 appear to be of special interest for several reasons, in particular because GLUT4 is directly regulated/stimulated by insulin and GLUT1 is not. It is found in various tissues like cardiac and skeletal muscles as well as adipose tissue [16]. The isolation and characterization of a monoclonal antibody that specifically recognises this "muscle-fat isoform" GLUT4 has revealed that it is 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 muscles [17].

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 2–3-fold increase in GLUT4 protein and gene expression in mixed soleus and gastrocnemius muscle preparations [18]. Exercise training also increases GLUT4 protein levels in the rat skeletal muscle [19], whereas there are conflicting results concerning patients with NIDDM: In the skeletal muscle, Handberg et al found no significant difference in the levels of GLUT4 mRNA and protein in biopsies from patients with and without NIDDM [20], whereas Dohm et al found a significantly decreased expression of GLUT4 in the skeletal muscle of insulin-resistant patients [21]. In rat cardiac tissue of streptozotocin-diabetic rats, Eckel and Reinauer showed that GLUT4 mRNA is decreased [22]. Interestingly, in the human heart, our own group showed that, in NIDDM patients, GLUT4 mRNA expression is down-regulated [23], whereas it is up-regulated in IDDM [24]. The latter may be explained by the fact that the application of insulin stimulates the expression of GLUT4 mRNA [17, 25]. The specific aim of the study is to elucidate effects of beta-adrenergic blockade on both the insulin-dependent glucose transporter GLUT4 and the insulin-independent GLUT1 in myocardial ischemia. We expect to learn from these results whether or not ischemic myocardial tissue from patients with and without beta-blockade shows altered patterns of metabolic gene expression profiles in order to improve understanding of anti-ischemic protection. In particular, we look at the expression of GLUT1 and GLUT4. In this context, 2 different beta blockers were tested.

Methods

We look at the effects of ischemia on mRNA expression in experimentally ischemic human myocardial tissue. Myocardial ischemia, in human tissue, leads to an altered molecular signature as we have published [4]. Results from molecular profiling during myocardial ischemia taken from preliminary experiments performed at the ZMF (Centre of Medical Research of the Medical University of Graz) indicate an involvement of beta blockers in anti-ischemic protection and appear to constitute a major controlling site of metabolism and cardio-protection in low-flow ischemia [5]. The present project is designed to study multiple gene expressions involved in myocardial metabolism under ischemic conditions using an appropriate experimental chamber, the PANTHER software was used in order to obtain specific understanding of possible pathways involved in response to ischemia and these shall be verified by real-time PCR (Light Cycler). We wished to see whether or not there is a difference in gene expression of metabolic pathways and processes in patients with and without beta-blockade under ischemic conditions. Doing so, we used human myocardial tissue from the right auricle of patients undergoing cardiac surgery. We studied 4 + 1 subjects in each group. Details of methods and a time table of experimental work are given below. Results were statistically evaluated in cooperation with the "Biostatistische Beratung ZMF" and the Institute of Medical Statistics and Biostatistics of the Medical University Graz. We expect to learn from these results whether or not ischemic myocardial tissue from patients with and without beta-blockade shows altered patterns of metabolic gene expression profiles in order to improve our understanding of anti-ischemic protection. In particular, we look at the expression of GLUT1 and GLUT4. Experimental techniques of ischemia have been well established by our group during the last 25 years in our group [2] and gene expression measurements have equally been established during our cooperation with Core Facilities Molecular Biology, ZMF, for several years and are constantly in use by our group [26]. There is a good experience of all coworkers involved in the on-going project.

1. Myocardial tissue probes will 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 extracorporal circulation and is normally wasted. The muscle piece will then be placed in cooled Tyrode solution and transported to the laboratory where it shall be placed into the experimental chamber as done in earlier experiments. The preparation will be oxygenated and then snap-frozen. The model allows for chemically and mechanically induced hypoxia/ischemia either by switching oxygen to nitrogen (hypoxia) or by using deoxy-glucose instead of glucose, both or by immersing the preparation in a layer of paraffin oil. Then, real-time GLUT4 PCR (Light Cycler) will be used, based on the works of Razeghi et al [4], Depre et al [27] and our own technical experience [28]. The combined technique of working on a living human preparation in an experimental chamber and the application of RT-PCR with a Light Cycler has been established in our laboratory of Experimental Cardiology over a period of several years in order to be able to answer the particular question of the interactions of hypertension, ischemia, and gene expression in the human heart. Here, the myocardial strip is instantly placed in well-oxygenated, cooled Tyrode solution and transported to the laboratory where it is fixed in an experimental chamber. The preparation then is oxygenated (100 % O_2) before it is cut in 2 pieces of about equal size. Each of the preparations is then put into another individual chamber as has been done in earlier experiments. Experimental ischemia is brought about by switching 100 % oxygen to 100 % nitrogen (hypoxia) in one of the chambers [29]. After 30 minutes of simulated

ischemia the tissue probes are snap-frozen using liquid nitrogen. By doing so, we are able to compare ischemic and non-ischemic tissues of the same patient. Snap-frozen samples are stored at –70 °C until RNA isolation. Preparation for RNA isolation is made by homogenisation of the paraffin-embedded probes using a cryostatic microtome (HM 560 CryoStar, Microm).

2. Solutions: The preparations are continuously perfused with Tyrode solution containing in mM: NaCl 140, KCI 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.0, glucose 10, HEPES 20. Solutions were adjusted to a pH of 7.4 by titration with 4M NaOH and equilibrated with 100 % O_2 at 37 °C [29].
3. RNA isolation and cDNA transcription: Total RNA is extracted by the Trizol® method (Invitrogen Corp, Carlsbad, CA, USA) and further purified using RNeasy Mini Kit (Qiagen Inc, Hilden, Germany). After drying, the pellet containing isolated RNA is re-suspended in approximately 30 μl TE buffer. Then the quality as well as quantity of RNA will be assessed using spectro-photometry. The quality of the isolated RNA is also analysed on Agilent's Bioanalyzer 2100 system. Either 20–40 μg or 0.2–1 μg total RNA, when only reduced amounts of material are available, is then directly or indirectly, via in vitro transcription, transcribed into DIG-labelled cDNA. For reverse transcription of isolated RNA we use the High Capacity cDNA Archive Kit (Applied Biosystems) and the Thermocycler MyCycler™ from Biorad. Real-time PCR shall be performed using the LightCycler® 2.0 System (Roche). Expression of genes is detected using the Taqman format and is mostly compared to the housekeeping gene glucose 6 phosphate dehydrogenase, which is measured using the hybridisation probe format with a kit from Roche (LightCycler-h-G6PDH Housekeeping Gene Set). We then use Taq DNA Polymerase for mastermix in both (LightCycler® DNA Master HybProbe). Forward primer (= Primer 1), reverse primer (= Primer 2) as well as the specific complementary Taqman probe are produced by TIB MolBiol Company. Then the expression ratio is calculated.
4. Microarray: The labelled probes are hybridised onto the array for 16 hrs. Subsequently, arrays are washed and detection is carried out using alkaline-phosphatase-conjugated anti-DIG antibodies and the appropriate substrate according to a highly standardized protocol. Arrays are scanned with the AB1700 Chemiluminescence Array Reader and images, raw data and tissue information is stored in a MIAME compliant ORACLE dat AB1700 Microarray Analyzer System: the full-genome Chemiluminescence Microarray System (Applied Biosystems) implemented at the Molecular Biology Core Facility of the Center for Medical Research combines the most comprehensive gene probe set with a chemiluminescence-based detection system that is superior to the commonly used fluorescent detection systems with respect to sensitivity (femtomolar level). Expression levels of up to 54,000 transcripts can be measured in one single experiment. About 74 % of the immobilized probes are CELERA-curated (CDS) sequences from public databases and 25 % of the sequences are accessible via the CDS only. About 4700 control spots are used to assess array performance in each single step from array fabrication to assay read-out. Another unique feature of the AB1700 system is the co-immobilization of an artificial oligonucleotide probe together with the gene-specific probe in each spot. This probe is detected via a fluorescently labelled oligo-nucleotide that is added to the hybridisation mixture. This novel approach is crucial for optimal localization of each spot and allows for normalization strategies that are not necessarily dependent on a high-end bio-informatics support [1, 4, 5]. In each experiment we use data pools of 4 experiments as well as one individual single experiment (n = 4 + 1).
5. Operator(s): The AB1700 microarray system available at the Core Facility Molecular Biology is operated by a team consisting of 4 members with many years of experience in various microarray technologies and platforms (Affymetrix, cDNA- and Oligonucleotide Arrays). To evaluate the performance of our Applied Biosystems microarray technology a multicenter proof-of-principle study was conducted by the CF-MB, which included Affymetrix, cDNA- and oligonucleotide-based platforms which was performed at approved international facilities. The novel AB1700 chemo-luminescence microarray system turned out to be superior with respect to sensitivity and reliability.
6. Solutions: The preparations are continuously perfused with Tyrode solution containing in mM: NaCl 140, KCl 4.5, $CaCl_2$ 2.5, $MgCl_2$ 1.0, glucose 10, HEPES 20. Solutions were adjusted to a pH of 7.4 by titration with 4 M NaOH and equilibrated with 100 % O_2 at 37 °C. Nebivolol experiments were performed using a 0.002269 molar stem solution (0.23 g nebivolol + 250 ml aqua dest) whereof 0.5 ml were added to 50 ml Tyrode which resulted in a 22.47 micromolar solution.

Results

SLC2A1 (GLUT1, solute carrier family 2 [facilitated glucose transporter], member 1) gene expression is significantly up-regulated by nebivolol and atenolol in well-oxygenated tissue but not during experimental myocardial ischemia:

Recently, our group investigated in samples from right auricles to which extent GLUT1 and GLUT4 gene expression is altered in the cardiac tissue of "healthy" subjects [10, 30]. We looked at the effects of experimental ischemia on and GLUT4 expression in the cardiac tissue of metabolically normal, non-obese, normotensive patients who underwent cardiac surgery for reasons like valve repair, trauma etc. We showed that GLUT1 and GLUT4 expressions remain unchanged or sometimes increase in the context of protecting myocardial metabolism secondary to increased glucose uptake through translocation of the transporters from an intracellular compartment to the sarcolemma. Using the microarray technique, we first looked at general changes in expression profiles during simulated myocardial ischemia, the behaviour of GLUT1+4 (solute carrier family 2 [facilitated glucose transporter], members 1 and 4) as well as their regulator genes SLC2A1RG and SLC2A4RG [31].

Then, using real-time PCR (Light Cycler), we quantified GLUT1 mRNA expression changes and investigated whether or not expression is significantly altered under ischemic conditions and which effect is brought about by atenolol and nebivolol. We found that, during experimental ischemia, there is an up-regulation of GLUT1 expression, however not statistically significant. This confirms earlier data by our group.

While there is no significant regulation with and without the influence of beta blockers during myocardial ischemia either, there is, however, a significant difference between the expression of GLUT1 in well-oxygenized preparations with (0.087 ± 0.02) and without nebivolol (0.62 ± 0.02; ± SEM; $p \leq 0.05$). Similarly, atenolol led to an increase of GLUT1 expression in well-oxygenated preparations compared to controls: 1.18 ± 0.08 and 0.62 ± 0.02, respectively (± SEM; $p < 0.05$) (Fig. 1).

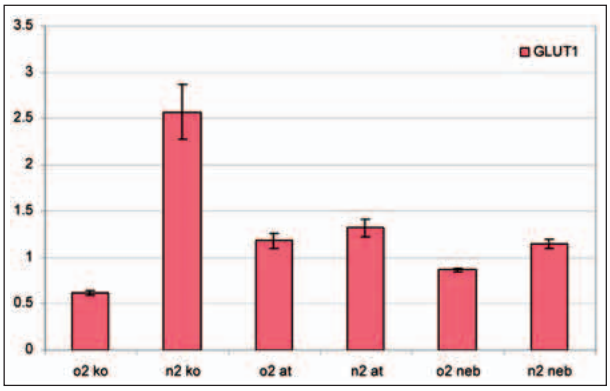

Figure 1. Results from real-time PCR measurements of 72 experiments (n = 12 in each group; o2ko = well-oxygenated, no ischemia, no drug; n2ko = experimental ischemia, no drug; o2at = well-oxygenated, no ischemia, atenolol present; n2at = experimental ischemia, atenolol present; o2neb = well-oxygenated, nebivolol present; n2neb = experimental ischemia, nebivolol present). It can be seen that during experimental ischemia, there is an up-regulation of GLUT1 expression, however, not statistically significant. This confirms earlier data by our group. While there is no significant regulation with and without the influence of beta blockers during myocardial ischemia either, there is, however, a significant difference between the expression of GLUT1 in well-oxygenized preparations with (0.87 ± 0.02) and without nebivolol (0.62 ± 0.02; ± SEM; $p \leq 0.05$). Similarly, atenolol led to an increase of GLUT1 expression in well-oxygenated preparations compared to controls: 1.18 ± 0.08 and 0.62 ± 0.02, respectively (+ SEM; $p < 0.05$).

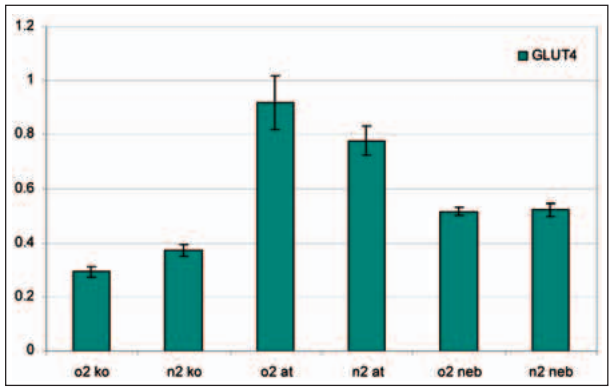

Figure 2. Results from real-time PCR measurements of all GLUT4 experiments (o2ko = well-oxygenated, no ischemia, no drug; n2ko = experimental ischemia, no drug; o2at = well-oxygenated, no ischemia, atenolol present; n2at = experimental ischemia, atenolol present; o2neb = well-oxygenated, nebivolol present; n2neb = experimental ischemia, nebivolol present). It can be seen that during experimental ischemia, there is an up-regulation of GLUT4 (SLC2A4) expression, however not statistically significant.

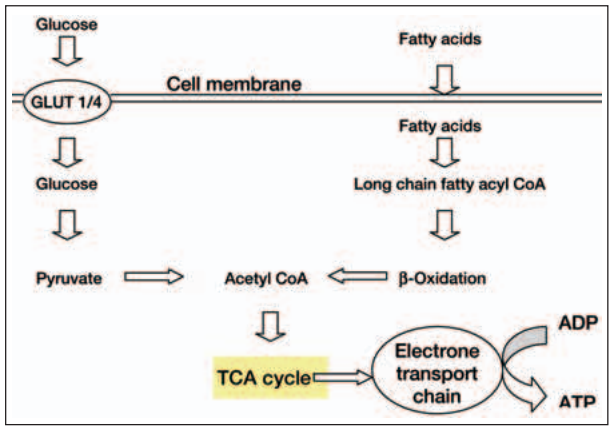

Figure 3. Substrates of myocardial metabolism. Mod. from [29, 32]. The fetal myocardial phenotype uses predominantly glucose for its metabolism, whereas the adult individual mainly metabolises fatty acids. During special conditions, like hypoxia, the adult phenotype of myocardial metabolism converts to the fetal phenotype, again preferably using glucose for its metabolism (Fig. 4). It has been shown that a preferentially glucose-oriented cardiac metabolism is beneficial in myocardial ischemia. However, knockout experiments have shown that successful transfer to the fetal metabolism is possible only under adequate/increased GLUT1 expression [33–35].

The above results may indicate that beta blockers could exert favorable metabolic effects upon myocardial tissue leading to enhanced trans-membrane glucose transport preparing the cell well for the maintenance of cellular metabolism during ischemia. In further experiments, we investigated the effect on GLUT4 (SLC2A4), the insulin-dependent transmembrane glucose transporter. These data are shown below.

SLC2A4 (GLUT4, solute carrier family 2 [facilitated glucose transporter], member 4) gene expression is significantly up-regulated by nebivolol and atenolol in well-oxygenated tissue but not during experimental myocardial ischemia:

Myocardial ischemia increases glucose uptake through translocation of GLUT1 and GLUT4 from an intracellular compartment to the 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. Delivery of glucose to the glycolytic pathway appears to be a major controlling site of glycolysis in low-flow ischemia. 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, little or nothing is known in this context about human cardiac transmembrane glucose transport, GLUT4 expression, and its regulation. According to GLUT1 we studied the effect of beta blockers on GLUT4 and were able to draw similar conclusions. Using the microarray technique, we find that both the expression of GLUT4 gene (SLC2A4) and its regulator gene remain practically unchanged. In real-time PCR (Light Cycler), the mean ratio for GLUT4 gene expression compared to the housekeeping gene G6PDH was as shown below.

No significant changes are seen in the expression of the GLUT4 gene as well as in its regulatory gene after 30 minutes of N_2-mediated experimental ischemia. Similarly, biological processes (microarray) involved in glucose metabolism are not as significantly de-regulated as are others. This, as well as a slight trend towards up-regulation, can be interpreted as an attempt of the myocyte to maintain its energy metabolism also under hypoxic conditions.

This confirms earlier data by our group. While there is no significant regulation with and without the influence of beta blockers during myocardial ischemia either, there is, however, a significant difference between the expression of GLUT4 in well-oxygenized preparations with (0.52 ± 0.01) and without nebivolol (0.29 ± 0.02; ± SEM; $p \leq 0.05$). Similarly, atenolol led to an increase of SLC2A4 expression in well-oxygenated preparations compared to controls: 0.92 ± 0.10 and 0.29 ± 0.02, respectively (+ SEM; $p < 0.05$) (Fig. 2).

Some of the anti-anginal effects of beta blockers may possibly be explained by their action on GLUT1 and GLUT4 expressions in myocardial cells, thus facilitating glucose uptake and in turn a shift to the fetal phenotype of metabolism.

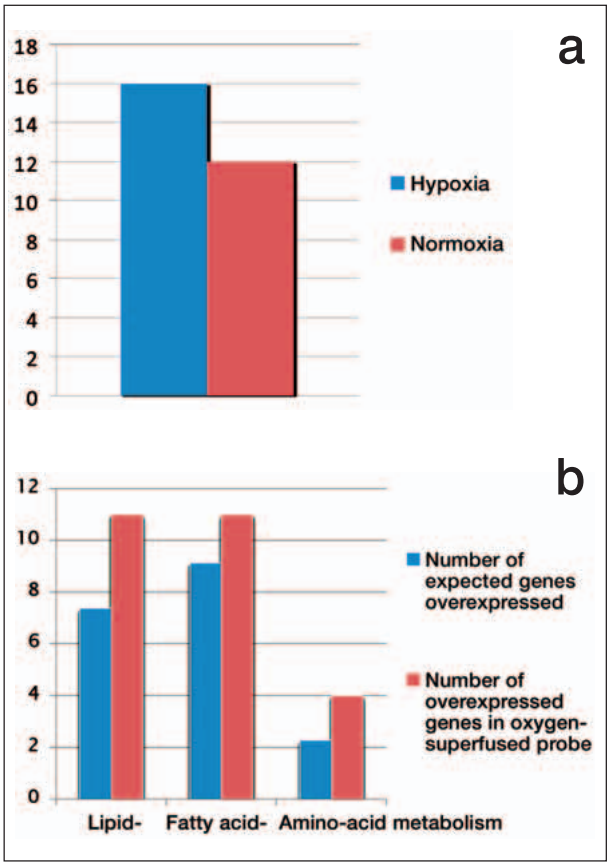

Figure 4. (a) Gene expression of biological processes associated with glucose metabolism during normoxia and hypoxia. Up-regulation of glucose metabolism. **(b)** Down-regulation of gene expression of biological processes associated with fatty acid and amino acid metabolism – (a) and (b) indicate a conversion to the fetal type of metabolism. Based on data from [4–9, 32–35].

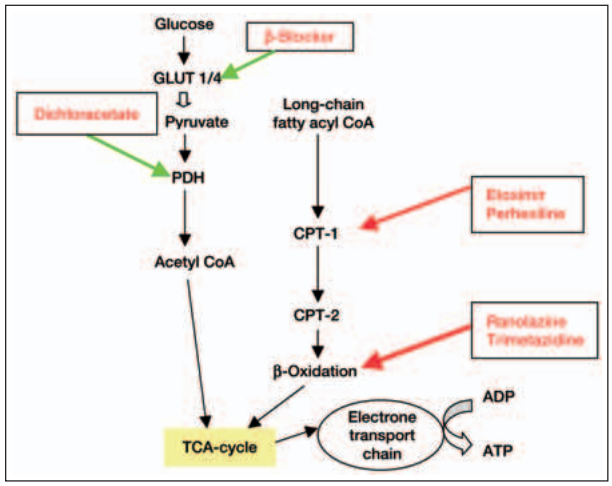

Figure 5. Shifting cardiac metabolism to its fetal phenotype: as an anti-anginal target (mod. from [32]) of dichloracetate augments cellular glucose metabolism. Etoximir and perhexiline inhibit cellular fatty acid metabolism and both ranolazine as well as trimetazidine slow down beta oxidation. Beta-adrenergic blockade enhances GLUT1 and GLUT4 expressions.

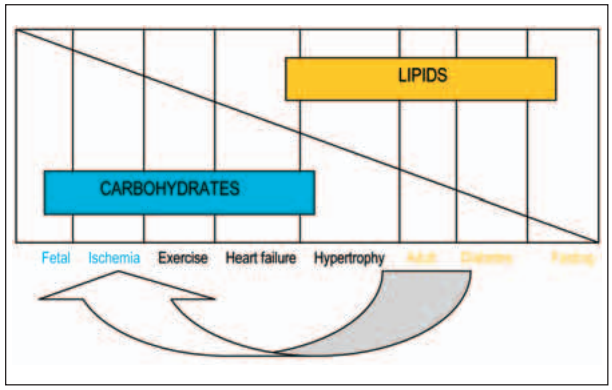

Figure 6. Plasticity of myocardial metabolism: during exercise, hypoxia/ischemia etc myocardial cells prefer glucose as a substrate. Both fasting and diabetes shift the metabolic substrates to the fatty acid site. Mod. from [29, 33].

Hypoxic/Ischemic Metabolism

Why are these data possibly of interest? As seen in Figure 3, the heart is able to use 3 different substrates for its metabolism – fatty acids, aminoacids, and glucose.

Our own microarray experiments confirm those data (Fig. 5). We find that gene expression of biological processes which are associated with glucose metabolism are up-regulated during hypoxia, whereas those associated with fatty acid and amino-acid metabolism are down-regulated (Fig. 5).

Do Nebivolol and Drugs Such As Ranolazin or Trimetazidine Show Synergistic Effects During Myocardial Ischemia?

Shifting myocardial metabolism to the fetal phenotype has become a new target for anti-anginal treatment. Figure 6 shows possibilities of shifting cardiac metabolism, either by augmentation of glucose metabolism or by inhibiting fatty acid metabolism. The latter has been successfully targeted by drugs like trimetazidine and ranolazine [32].

All these relatively new anti-anginal drugs are designed to shift adult towards fetal metabolism in cardiomyocytes in order to improve metabolism during ischemia. Some of the anti-anginal effects of beta blockers may possibly be explained by their action on GLUT1 expression in myocardial cells, thus facilitating glucose uptake and in turn a shift to the fetal phenotype of metabolism.

Discussion

Glucose Metabolism

The interrelation between disturbances in glucose metabolism, hypertertrophy, and myocadial ischemic heart 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 [26, 36–45]. 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 [45], non-diabetic patients with essential hypertension (leading to myocardial hypertrophy) and seem to be largely responsible for the development of hypertension. The common coexistence 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 hypertension, inappropriate vasoconstriction, structural changes of the cardiovascular system [46–48] as to its stiffness, but also unfavorable distribution of liquid between the compartments play a key role. While the complete cascade of interactions between glucose metabolism, hypertension, and cardiac hypertrophy has not been as yet completely elucidated, trans-membrane glucose transport is certainly crucial in this setting [18–21, 26]. In the present project, we wish to intensify our investigational efforts focused on the unknown role played by GLUT4 in the development of myocardial hypertrophy. 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 trans-membrane glucose transport in various tissues. GLUT1 appears to be of special interest for several reasons, in particular, because it is not directly regulated/stimulated by insulin and keeps up the background glucose supply of the cell. It is found in various tissues such as cardiac and skeletal muscles, as well as adipose tissue. The isolation and characterisation of a monoclonal antibody that specifically recognised this isoform GLUT1 has revealed that it was a unique isoform, different from other glucose transporters.

Increased glucose uptake and metabolism by the ischemic myocardium help protect myocardial cells from irreversible injury (see below), but this may be only half the truth. In particular, intracellular acidification and subsequent Ca-overload may result from excessive anaerobic metabolism and reperfusion during early ischemia (intracellular protons, primarily generated during anaerobic metabolism, are then extruded from the cell via the Na^+/H^+ carrier in exchange for Na^+ leading to an accumulation of Na^+ ions in the cytoplasm). The myocardial cell, attempting to maintain the transmembrane Nernstian and Goldmanian ionic equilibrium, extrudes the excess Na^+ ions using the reverse mode NCX, thus causing intracellular Ca^{++} overload with the known consequences leading to myocardial stunning and in extension to cell death. Possibly, it is the timely sufficient support with glucose from which the cell benefits.

GLUT4 and Myocardial Ischemia

Glucose and high-energy metabolisms 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 [2, 3, 49, 50]. Many of these aspects of myocardial ischemia are linked in one way or the other to transmembrane glucose transport, intracellular glucose metabolism and, in fact, to GLUT4 [51–53]. Myocardial ischemia increases glucose uptake through translocation of GLUT1 and GLUT4 from an intracellular compartment to the sarcolemma. This appears to be a beneficial effect during ischemia and possible recovery. Insulin and ischemia have additive effects to increase in vivo glucose utilisation and augment glucose transporter translocation [54]. 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 [55]. 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 [563], only recently data from human ischemic tissue became available, surprisingly indicating little to no change in GLUT4 expression during ischemia [2, 3, 7, 31, 49]. Both GLUT4 mRNA expression of myocardial GLUT4 (SLC2A4) and its regulator gene SLC2A4RG remain unaffected during experimental hypoxia, possibly a limiting factor for protection and recovery [31]. In contrast, GLUT4 expression is increased during ischemia in hypertensive subjects [9]. From both our experiments and the literature one can see that during myocardial ischemia a shift from fatty acid (adult) metabolism to a more glucose-based (fetal) metabolism occurs in order to provide sufficient energy. Thus, to some extent myocardial integrity is preserved for some time. Modern drug developments have addressed this observation and, as can be seen in Figure 6, several drugs exist which support this metabolic shift. These drugs, especially ranolazine, which are used in anti-anginal therapy, are able to induce a switch of the metabolic substrate [32, 56]. The alternative pathway, or, in other terms, the fetal phenotype of metabolism, is induced either by augmentation of glucose metabolism or by inhibiting the fatty acid metabolism. The latter has been successfully targeted by drugs like trimetazidine and ranolazine [32]. Here we show for the first time that some of the anti-anginal effects of beta blockers may be brought about by their action on GLUT1/4 expression in myocardial cells, thus facilitating glucose metabolism and, in turn, causing a shift to the fatal phenotype of metabolism in the adult human heart. The fetal myocardial phenotype uses predominantly glucose for its metabolism, whereas the adult individual mainly metabolises fatty acids, as can be seen in Figure 3. During special conditions, like hypoxia, the adult phenotype of myocardial metabolism converts to the fetal phenotype, again preferably using glucose for its metabolism (Fig. 4). It has been shown that a preferentially glucose-oriented cardiac metabolism is beneficial in myocardial ischemia. However, knockout experiments have shown that successful transfer to the fetal metabolism is possible only under adequate/increased GLUT1 expression [57]. Here we show for the first time that the expression of both GLUT1 and GLUT4 is augmented by beta blockers, thus enabling increased glucose transport across the membrane. The increased insulin-dependent and -independent glucose uptake brought about by beta blockers may thus constitute an important synergistic effect with modern anti-anginal drugs which induce the preferential use of glucose as a substrate of cardiac metabolism.

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