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## Effect of Betablockers on the Regulation of PDK (Pyruvate Dehydrogenase Kinase) Gene Expression in Both Normoxic and Hypoxic Myocardium

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*Pyruvate dehydrogenase kinase isoforms inhibit pyruvate dehydrogenase, which constitutes an important step in glucose metabolism. It is involved in various phenomena of aging and its expression changes with age – a mechanism that is so far not well-understood. Cardiac metabolism of glucose is very tightly controlled in order to maintain the variable energy demand that is required by cardiac tissue. Energy metabolism of the cardiac myocyte can be regulated within seconds up to a few minutes or chronically regulated within the time frame of hours to days. Glucose metabolism is activated in early myocardial ischemia and in response to an increased need of high-energy phosphate in the healthy heart during extreme physical activity. In myocardial ischemia, inhibition of PDK expression would be beneficial in order to shift the myocardial metabolism from the adult towards the fetal phenotype, thus metabolising more glucose than fat to preserve myocardial integrity.*

*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 extra-corporeal circulation. This sample is then placed in cooled Tyrode solution and hypoxia is brought about by switching 100 % oxygen to 100 % nitrogen (hypoxia) in one of the 2 chambers. By doing so, we are able to compare ischemic and non-ischemic tissues of the same patient. Snap-frozen samples are stored at -170 °C until RNA isolation. Quality of isolated RNA is analysed by means of the Agilent's Bioanalyzer 2100 system. Arrays are scanned with the AB1700 Chemiluminescence Array Reader and images as well as data are processed using the PANTHER software.*

*In our microarray experiments, we find that, in particular, PDK isoform 4 is significantly less expressed under nebivolol both during O<sub>2</sub> perfusion and simulated ischemia, an effect practically negligible under atenolol. Here, nebivolol also exhibits a unique cardioprotective property different from standard betablockers.*

*We find that without the influence of betablockers there is no significant regulation of PDK expression during myocardial ischemia. There is merely a trend towards a decrease in PDK gene expression. There is, however a significant difference between the expression of PDK during myocardial ischemia in the presence of atenolol ( $3.62 \pm 0.18$ ) and nebivolol ( $1.97 \pm 0.06$ ; + SEM;  $P \leq 0.05$ ): PDK expression is decreased during normoxia (trend) and ischemia (significant) in the presence of nebivolol.*

*Here, confirmed by real-time PCR, the finding that PDK gene expression is down-regulated by nebivolol compared to atenolol in normoxia (trend, not statistically significant) and simulated ischemia/hypoxia (statistically significant) may argue for a higher protective, anti-ischemic but also anti-anginal metabolic potential of nebivolol compared to standard betablockers like atenolol. Especially patients with angina may profit from this particular property of nebivolol over atenolol. J Clin Basic Cardiol 2010; 13: 12–8.*

**Key words:** betablockers, PDK, heart, metabolism

Little is known about the molecular effects of betablockers upon myocardial ischemia. In earlier research, we identified the specific molecular signature of myocardial ischemia, which, on the one hand, may represent the severity and type of tissue damage produced; on the other hand, it demonstrates the activation of repair mechanisms and changes in the metabolic state of the cell. Doing so in the presence and absence of the betablockers nebivolol and atenolol now allows for conclusions in which direction cardioprotective mechanisms of the drug should be further investigated. In particular, we studied pyruvate dehydrogenase kinase (PDK), an important enzyme in the regulation of the conversion of pyruvate to acetyl-CoA in mammals [1]. Glucose oxidation depends on the flux through the pyruvate dehydrogenase complex (PDC), a mitochondrial enzyme complex. PDK phosphorylates PDC and as a consequence it is inactive. On the other hand, pyruvate dehydrogenase phosphatase dephosphorylates and as a result reactivates the PDC [2].

Cardiac metabolism of glucose is very tightly regulated to maintain the variable energy demand required by the cardiac

tissue. Energy metabolism of the cardiac myocyte can be regulated within seconds up to a few minutes or chronically regulated within the time frame of hours to days. Glucose metabolism is activated in early myocardial ischemia – a sensitive response to the increased need of high-energy phosphate in the healthy heart during extreme physical activity [3]. However, in coronary heart disease, this activation becomes deleterious. Metabolism of glucose in the cardiac myocyte occurs in 2 stages: (1) glycolysis converts glucose to pyruvate in cytosol and (2) glucose oxidation encompasses the metabolism of mitochondrial pyruvate to acetyl CoA and entry into the citric acid cycle. The rate of glucose oxidation is dependent on flux through the pyruvate dehydrogenase complex (PDC) and mitochondrial enzyme complex, which converts pyruvate to acetyl CoA. Phosphorylation of PDC by the associated enzyme pyruvate dehydrogenase kinase (PDK) inactivates the PDC complex, whereas pyruvate dehydrogenase phosphatase dephosphorylates and reactivates the enzyme. Activation of PDK phosphorylates and thus inhibits PDC, thereby decreasing glucose oxidation. Increased pyruvate supply can inhibit PDK, thereby stimulating PDC, as

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seen in isolated insulin-perfused hearts. In myocardial ischemia, inhibition or decreased gene expression of PDK is necessary to shift the myocardial metabolism towards the fetal phenotype, thus metabolising more glucose than fat [4, 5] in order to preserve myocardial integrity. Dichloroacetate, an inhibitor of PDK and thus a stimulator of PDH, has been already suggested as an anti-anginal target drug [2].

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 so far not been looked at in human myocardial tissue in depth. Hence, it remains unknown whether or not ischemia can be related to a specific pattern of metabolic gene expression. The latter, in form of a particular molecular signature, reveals a new understanding of cardioprotective mechanisms. Using this technique, relevant cardioprotective properties of anti-ischemic drugs can be more easily accessed. In order to gain more understanding of the relevant molecular pathways and biochemical processes, the effect of betablockers was looked at in this project. Betablockers, known for their anti-ischemic properties, affect certain pathways and biological processes during simulated ischemia, which can then be identified.

Different biochemical variants of betablockers entail different anti-ischemic actions which can be identified at the level of gene expression.

We looked at the effects of ischemia on mRNA expression in experimentally ischemic human myocardial tissue. In human tissue, myocardial ischemia leads to an altered molecular signature as shown in [6–10]. Results from molecular profiling during myocardial ischemia taken from preliminary experiments indicated an involvement of betablockers in anti-ischemic protection and appear to be a major controlling site of metabolism and cardioprotection in low-flow ischemia. While many experimental studies suggest that changes in gene expression within the ischemic myocardium help to protect myocardial cells from irreversible injury, little is known in this context about human cardiac ischemic gene expression and the interrelation between the latter and cardioprotective agents like betablockers.

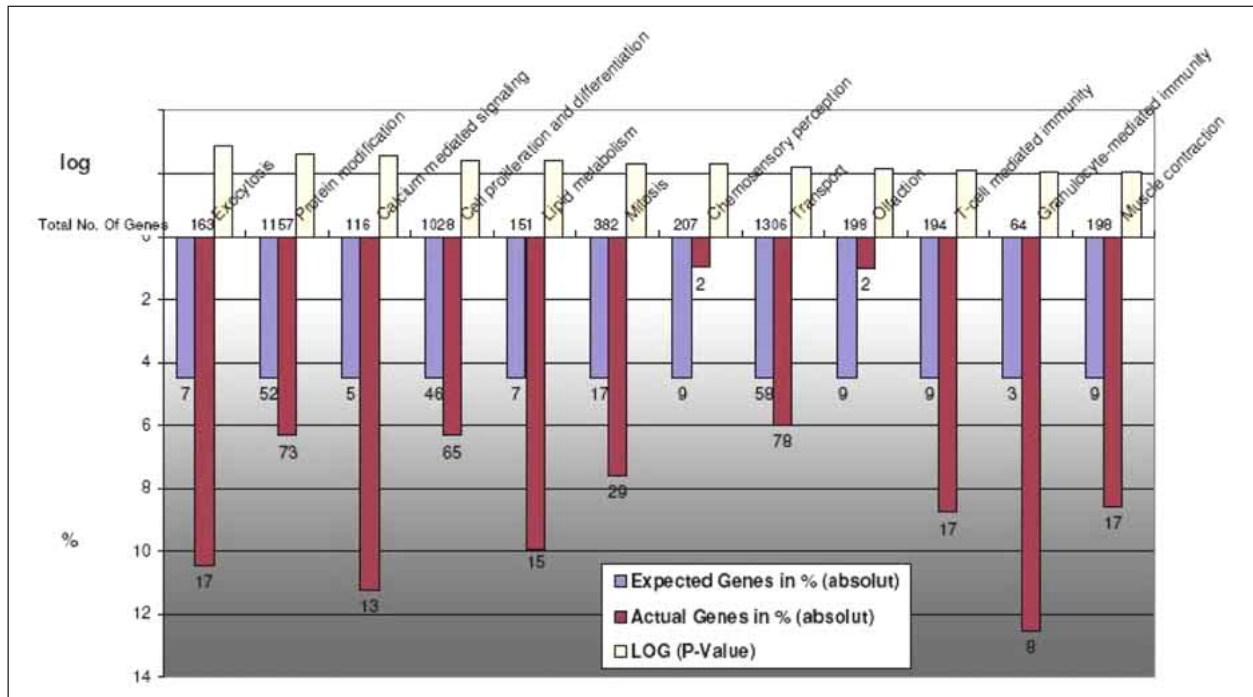
The present project was designed to study multiple gene expressions under ischemic conditions using an appropriate experimental chamber (see methods), the PANTHER software was used to obtain specific understanding of possible pathways involved in response to ischemia and these were verified by real-time PCR (Light Cycler) experiments. We elaborated characteristic differences in gene expression of patients with and without betablockade under ischemic conditions. Doing so, we used human myocardial tissue from the right auricle of patients undergoing cardiac surgery. We studied 10–12 subjects in each group (PCR). We learned from these results that ischemic myocardial tissue from patients with and without betablockade shows differential patterns of gene expression profiles [6–10]. In the long run, these results will improve the understanding of anti-ischemic protection. In this context, the betablockers nebivolol and atenolol were tested.

Here, we look at mRNA expression of PDK during myocardial ischemia in the presence of various betablockers. We find that, without the influence of betablockers, there is no significant regulation of PDK expression during myocardial ischemia. We also find that PDK expression is decreased during normoxia (trend) and ischemia (significant) in the presence of nebivolol (not seen with atenolol). This would activate the carbohydrate-based metabolism and thus possibly protect from ischemic injury.

## 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 showed in [11]. 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 betablockers in anti-ischemic protection and appear to constitute a major controlling site of metabolism and cardio-protection in low-flow ischemia [6]. The present project is designed to study multiple gene expressions involved in myocardial metabolism under ischemic conditions using an appropriate experimental chamber (see “Methods”), the PANTHER software was used 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 betablockade 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 (microarray). Details of methods and time table of experimental work can be seen 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 betablockade shows altered patterns of metabolic gene expression profiles to improve our understanding of anti-ischemic protection. In particular, we look at the expression of GLUT 1 and GLUT 4. In this context, 2 different betablockers were tested. Details of methods and time table of experimental work can be seen below. Experimental techniques of ischemia have been well-established by our group over the last 25 years [12] and gene expression measurements [13] have equally been established during our cooperation with Core Facilities Molecular Biology, ZMF, for several years and are constantly in use. There is a good experience of all co-workers involved in the ongoing project.

a) 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 extracorporeal 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 perfusing with 100 % oxygen (control). Then, real-time PCR (Light Cycler) will be used, based on the works of Schmittgen et al [14], Depre et al [15], and Livak et al [16] and our own technical experience. The combined technique of working on a living human preparation in an experimental chamber and the application of real-time 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<sub>2</sub>) before it is cut in 2



**Figure 1.** Example of differential regulation of gene expression by atenolol and nebivolol in well-oxygenized preparations: one can see that numerous biochemical processes are affected by nebivolol but not by atenolol. For example, many processes involved in contraction, lipid metabolism, and proliferation are down-regulated by nebivolol only (not by atenolol). Reducing lipid metabolism in exchange for an increased carbohydrate metabolism may render the heart less vulnerable to O<sub>2</sub> deficit or ischemia.

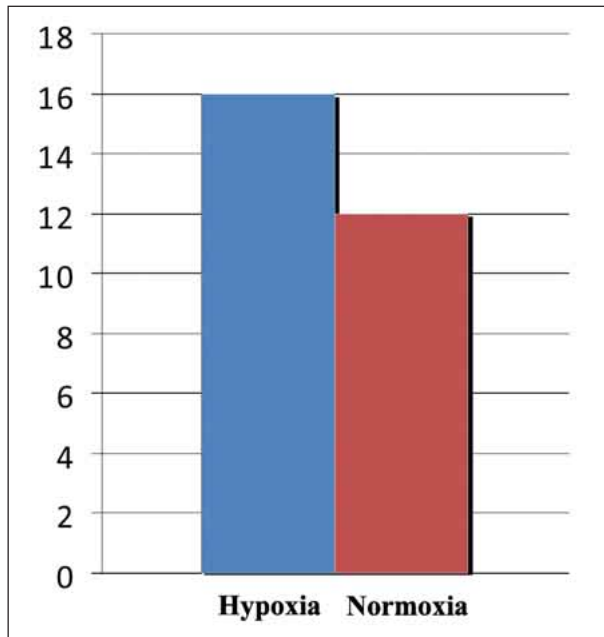
halves of about equal size. Each of the preparations is then put into another individual chamber as done in earlier experiments. Experimental ischemia is brought about by switching 100 % oxygen to 100 % nitrogen (hypoxia) in one of the chambers [17]. After 30 minutes of simulated ischemia the tissue 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 -170 °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).

- b) Solutions: preparations are continuously perfused with Tyrode solution containing (in mM): NaCl 140, KCl 4.5, CaCl<sub>2</sub> 2.5, MgCl<sub>2</sub> 1.0, glucose 10, and HEPES 20. Solutions were adjusted to a pH of 7.4 by titration with 4 M NaOH and equilibrated with 100 % O<sub>2</sub> at 37 °C [18]. 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 resulting in a 22.47-micromolar solution.
- c) RNA isolation and cDNA transcription [14, 15]: total RNA is extracted by means of the TRIZOL<sup>®</sup> method (Invitrogen Corp, Carlsbad, CA, USA) and further purified using the RNeasy Mini Kit (QIAGEN Inc, Hilden, Germany). After drying, the pellet containing isolated RNA is re-suspended in approximately 30 µl TE buffer. Then quality as well as quantity of RNA was 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 (Ap-

plied Biosystems) and the Thermocycler MyCycler<sup>™</sup> (Biorad). Real-time PCR shall be performed using the LightCycler<sup>®</sup> 2.0 System (Roche). Expression of genes is detected using the Taqman format and is mostly compared to the house-keeping 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<sup>®</sup> 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.

- d) Microarray [13]: 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 are 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 CELERACURATED (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.





**Figure 2.** Gene expression of biological processes associated with glucose metabolism during normoxia and hypoxia is down-regulated in human atrial tissue. Up-regulation of gene-expression associated with glucose metabolism during hypoxia.

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 normalization strategies that do not necessarily depend on a high-end bio-informatics support. In each experiment, we use pooled data from 4 experiments as well as one individual single experiment ( $n = 4 + 1$ ). The PANTHER software was used to process the results statistically and according to biological pathways and biochemical processes [19].

- e) Operator/s: The AB1700 microarray system available at the Core Facility Molecular Biology is operated by a team of 4 with many years of experience in various microarray

technologies and platforms (Affymetrix, cDNA- and Oligonucleotide Arrays) [20]. To evaluate the performance of our Applied Biosystems microarray technology a multi-center proof-of-principle study was conducted by the CF-MB, which included Affymetrix, cDNA- and oligonucleotide-based platforms and that was performed at approved international facilities. The novel AB1700 chemo-luminescence microarray system turned out to be superior with respect to sensitivity and reliability.

- f) Ethics committee: the study is in accordance with good clinical and scientific practice. It was approved by the local ethics committee. All patients who underwent cardiac surgery gave informed consent that the small part of their right auricle, which would normally have been wasted, could be used for scientific investigation for this specific study.

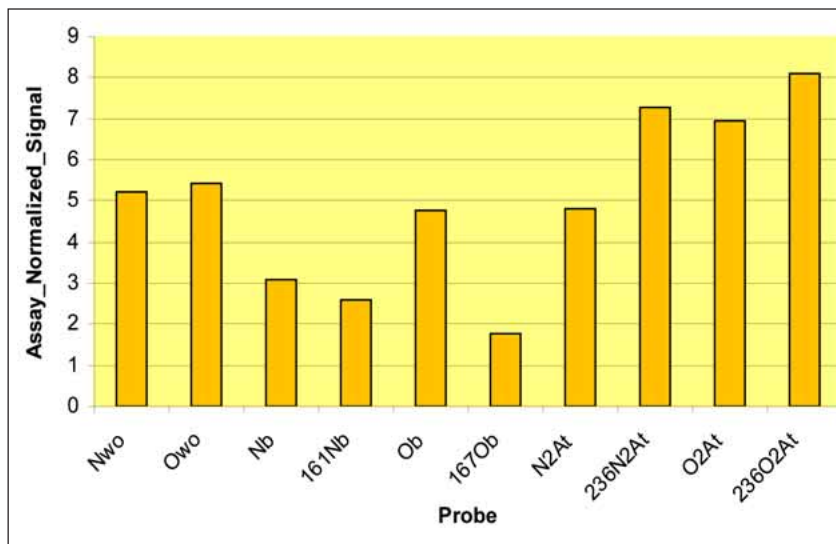
## Results

In our microarray experiments, we find a differential regulation of gene expression by atenolol and nebivolol in well-oxygenized preparations. Numerous biochemical processes are affected by nebivolol but not by atenolol. Figure 1 indicates that processes involved in contraction, lipid metabolism, and proliferation are down-regulated by nebivolol only (not by atenolol). Reducing the lipid metabolism in exchange for an increased carbohydrate metabolism may render the heart less vulnerable to  $O_2$  deficits or ischemia.

In our microarray experiments, we find that gene expression of biological processes associated with glucose metabolism during normoxia and hypoxia is down-regulated, as can be seen in Figure 2 (up-regulation of gene expression associated with glucose metabolism during hypoxia).

It can be seen that, without the influence of betablockers, there is no significant regulation of PDK expression during myocardial ischemia. While there is no down-regulation of PDK gene expression by atenolol, there is a decrease in PDK gene expression in the presence of nebivolol both during normoxia and hypoxia, as can be seen in Figure 3, which illustrates results from microarray experiments.

PDK is significantly less expressed under nebivolol both during  $O_2$  perfusion and simulated ischemia, an effect practically negligible under atenolol. Here, nebivolol also exhibits a unique cardioprotective property different from standard betablockers.



**Figure 3.** In this figure, the results from microarray measurements of PDK experiments are illustrated. Pooled DNA data: **Control experiments:** Owo: pool control, well-oxygenated, no ischemia, no drug; Nwo: pool experimental ischemia, no drug; **Atenolol experiments:** O2At: pool well-oxygenated, no ischemia, atenolol present; N2At: pool experimental ischemia, atenolol present; **Nebivolol experiments:** Ob: pool well-oxygenated, nebivolol present; Nb: pool experimental ischemia, nebivolol present. It can be seen that, without the influence of betablockers, there is no significant regulation of PDK expression during myocardial ischemia. While there is no down-regulation of PDK gene expression by atenolol, there is a decrease in PDK gene expression in the presence of nebivolol both during normoxia and hypoxia. 167Nb, 167Ob, 236N2At and 236O2At are corresponding single-experiment controls.

Using real-time PCR, we evaluated PDK mRNA expression changes and investigated whether or not gene expression is significantly down-regulated by nebivolol compared to atenolol under ischemic conditions. We found that, during experimental ischemia, there is a significant down-regulation of PDK 4 expression (Fig. 4). Without the influence of beta-blockers, there is no significant regulation of PDK expression during myocardial ischemia. There is, however, a significant difference between the expression of PDK during myocardial ischemia in the presence of atenolol ( $3.62 \pm 0.18$ ) and nebivolol ( $1.97 \pm 0.06$ ;  $\pm$  SEM;  $P \leq 0.05$ ).

Here, confirmed by real-time PCR, the finding that PDK gene expression is down-regulated by nebivolol compared to atenolol in normoxia (trend, not statistically significant) and simulated ischemia/hypoxia (statistically significant) may argue for a higher protective, anti-ischemic but also anti-anginal metabolic potential of nebivolol compared to standard beta-blockers like atenolol. Especially patients with angina may profit from this particular property of nebivolol over atenolol.

### Discussion

While drugs exert specific class effects within one particular class of drug, each class member may entail additional specific properties. This effect was called "dirty drug effect" in the past. However, more recently these effects have attracted the increased attention of scientists. An ideal example is the non-lipid-related effect of statins which has led to their far more extended application. The complexity of the inflammatory cascade involved in the development of atherosclerosis makes it difficult to develop single-target drugs in order to slow or impact the process. Drug researchers are always in search for new indications and atherosclerosis has been a target for a long time. Just very recently, it has been shown that inflammation *per se* could be an attractive target for pharmacological intervention in primary prevention. The JUPITER trial directly addressed this problem: in apparently healthy persons without hyperlipidemia but with elevated high-sensitivity C-reactive protein levels, a statin significantly reduced the incidence of major cardiovascular events by unfolding pleiotropic anti-inflammatory actions. Similar effects have recently also been discussed for beta-blockers. The latter constitute a rather heterogeneous group of drugs.

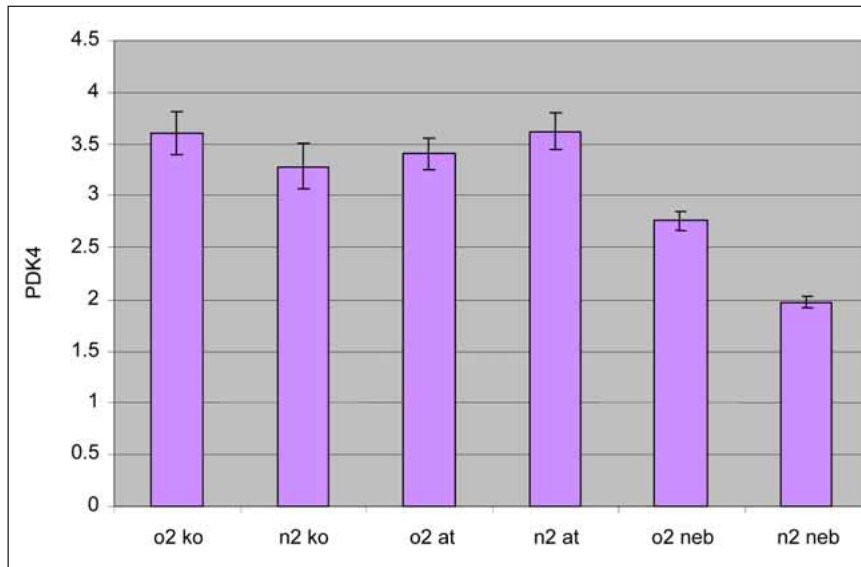
For example, nebivolol constitutes a special type of beta-blocker, which shows properties different from those of other beta-blockers [21, 22]. It exerts various additional effects, such as acting on the endothelial NO-pathway [23, 24] and an extremely high affinity to the  $\beta_1$  and  $\beta_3$  adrenoceptors. It also shows a high affinity to serotonin receptors of the 5-HT<sub>1A</sub> subtype and has been attributed a considerable cardioprotective effect [25, 26]. During and after myocardial ischemia, beta-blockers support the preservation of myocardial integrity. Since nebivolol shows numerous pharmacological properties which may entail anti-ischemic protection, we analysed the expression of 27,184 genes in human atrial myocardium under ischemic conditions in the presence and absence of nebivolol and compared it to atenolol. However, little is known about the molecular effects of nebivolol upon myocardial ischemia [27, 28]. In the present work, we identified the specific molecular signature of beta-blockers in myocardial ischemia, which, on the one hand, represents the severity and type of tissue damage produced. On the other hand, it demonstrates the activation of repair mechanisms and changes in the metabolic state of the cell. Doing so in the presence and absence of nebivolol and atenolol will allow for conclusions in which direction cardioprotective mechanisms of the drug should be further investigated.

Quality as well as quantity of isolated mRNA were double-checked using both spectrophotometry as well as the Agilent's Bioanalyzer 2100 system. The AB1700 microarray system available at the Core Facility Molecular Biology is operated by a team of 4 with many years of experience in various microarray technologies and platforms (Affymetrix, cDNA and Oligonucleotide Arrays). To evaluate the performance of the novel Applied Biosystems microarray technology a multicenter proof-of-principle study was conducted by the CF-MB, which included Affymetrix, cDNA- and oligonucleotide-based platforms and was performed at approved international facilities. The novel AB1700 chemiluminescence microarray system turned out to be superior with respect to sensitivity and reliability. Hence, we believe that data are highly reliable.

One can see that, in the presence of nebivolol, processes that are mainly involved in myocardial ischemia, damage, and inflammation are down-regulated, while processes involved in structural integrity, circulation, gas exchange activity, glucose homeostasis, and other vital cellular mechanisms expressing myocardial anti-ischemic protection are significantly more expressed. From these data one can conclude that nebivolol exerts an important cardioprotective action on a variety of intracellular pathways.

The present study verified one important molecule, PDK, by real-time PCR. Nebivolol was compared with atenolol, another standard beta-blocker commonly used in the treatment of myocardial ischemia, and we found remarkable differences in the expression profiles between different types of beta-blockers during myocardial ischemia. We also investigated whether or not nebivolol unfolds cardioprotective actions different from other beta-blockers upon the glucose metabolism: the interrelation between disturbances in glucose metabolism, hypertrophy, and myocardial 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 [29–38]. 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 [38], 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 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 [39–41] with regard to its stiffness, but also unfavourable distribution of liquid between the compartments play a key role. Glucose oxidation depends on the flux through the pyruvate dehydrogenase complex (PDC), a mitochondrial enzyme complex. PDK phosphorylates PDC and, as a consequence, it is inactive. On the other hand, pyruvate dehydrogenase phosphatase dephosphorylates and, as a result, reactivates the PDC [2].

The cardiac metabolism of glucose is very tightly regulated to maintain the variable energy demand that is required by the cardiac tissue. The energy metabolism of the cardiac myocyte can be regulated within seconds up to a few minutes or chronically regulated within the time frame of hours to days. The glucose metabolism is activated in early myocardial ischemia – a sensitive response to the increased need of high-energy phosphate in the healthy heart during extreme physical activity [3]. However, in coronary heart disease, this activation becomes deleterious. Metabolism of glucose in the



**Figure 4.** The results from real-time PCR measurements of PDK experiments are illustrated (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, without the influence of betablockers, there is no significant regulation of PDK expression during myocardial ischemia. There is just a trend towards a decrease in PDK gene expression. There is, however, a significant difference between the expression of PDK during myocardial ischemia in the presence of atenolol ( $3.62 \pm 0.18$ ) and nebivolol ( $1.97 \pm 0.06$ ;  $\pm$  SEM;  $P \leq 0.05$ ): PDK expression is decreased during normoxia (trend) and ischemia (significant) in the presence of nebivolol.

cardiac myocyte occurs in 2 stages: (1) glycolysis converts glucose to pyruvate in cytosol and (2) glucose oxidation encompasses the metabolism of mitochondrial pyruvate to acetyl CoA and entry into the citric acid cycle. The rate of glucose oxidation depends on the flux through the pyruvate dehydrogenase complex (PDC) and mitochondrial enzyme complex, which converts pyruvate to acetyl CoA. Phosphorylation of PDC by the associated enzyme pyruvate dehydrogenase kinase (PDK) inactivates the PDC complex, whereas pyruvate dehydrogenase phosphatase dephosphorylates and reactivates the enzyme. Activation of PDK phosphorylates and thus inhibits PDC, thereby decreasing glucose oxidation. Increased pyruvate supply can inhibit PDK, thereby stimulating PDC, as seen in isolated, insulin-perfused hearts. In myocardial ischemia, inhibition or decreased gene expression of PDK is necessary to shift the myocardial metabolism towards the fetal phenotype, thus metabolising more glucose than fat [4, 5] in order to preserve myocardial integrity. Dichloroacetate, an inhibitor of PDK and thus a stimulator of PDH has already been suggested as an anti-anginal target drug [2].

Increased glucose uptake and metabolism by the ischemic myocardium help to 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  $\text{Na}^+/\text{H}^+$  carrier in exchange for  $\text{Na}^+$  leading to an accumulation of  $\text{Na}^+$ -ions in the cytoplasm). The myocardial cell, attempting to maintain the transmembrane Nernstian and Goldmanian ionic equilibrium, extrudes the excess  $\text{Na}^+$ -ions using the reverse mode NCX, thus causing intracellular  $\text{Ca}^{++}$ -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. During special conditions, like hypoxia, the adult phenotype of myocardial metabolism converts to the fetal phenotype, again preferably using glucose for its metabolism (Fig. 2). It has been shown that a preferentially glucose-oriented cardiac metabolism is beneficial in myocardial ischemia. However, knock-out experiments have shown that successful transfer to fetal metabolism is possible only under adequate/increased

GLUT 1 expression [3, 42]. We show for the first time, confirmed by real-time PCR, that PDK gene expression is down-regulated by nebivolol compared to atenolol in normoxia (trend, not statistically significant) and simulated ischemia/hypoxia (statistically significant). This may argue for a higher protective, anti-ischemic but also anti-anginal metabolic potential of nebivolol compared to standard betablockers like atenolol. Especially patients with angina may profit from this particular property of nebivolol over atenolol.

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