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Differential Gene Expression under Nebivolol and Atenolol during Experimental Ischemia in Human Myocardium

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Introduction: During and after myocardial ischemia, betablockers support the preservation of myocardial integrity. In the present study, nebivolol is compared with another standard betablocker, here atenolol, commonly used in the treatment of myocardial ischemia. In human preparations, we found remarkable differences in the expression profiles between nebivolol and atenolol, both during simulated myocardial ischemia and normoxia. Here we report on these noteworthy differences in expression profiles. Results: Our results show that, in particular, on the level of reverse transcription and T-cell-mediated immunity as well as in the process of angiogenesis, a noteworthy regulation is brought about by nebivolol. Similarly, biological processes involved in cell-mediated immunity and reverse transcription are significantly up-regulated by nebivolol and not by atenolol. Cell-mediated immunity is largely involved in tissue repair. In well-oxygenized preparations, we have shown that numerous biochemical processes, mainly those involved in signalling and cellular immunity, are affected by nebivolol but not by atenolol. This shows that, in non-ischemic, well-perfused preparations, nebivolol unfolds particular, possibly cardioprotective effects which cannot be observed under atenolol. Similarly, we can see that many processes involved in contraction, lipid metabolism, and proliferation are down-regulated by nebivolol only (not by atenolol). In well-oxygenized preparations, we found numerous biochemical processes up-regulated by nebivolol (not by atenolol): of special interest in this context are reverse transcription, stress response, and protein phosphorylation. It is interesting that nebivolol, but not atenolol, leads to a down-regulation of gene expression in glutamine-glutamate conversion and pyruvate metabolism, the latter of particular interest in ischemia. In well-oxygenized preparations, we can see that numerous biological pathways, mainly those involved in signalling, angiogenesis, cellular immunity, and EGF are affected by nebivolol but not by atenolol. Concerning nebivolol-regulated biological pathways, we could see that, during normoxia, up-regulation of pathways is hardly pronounced by nebivolol over atenolol. From these data we can certainly deduce that nebivolol unfolds a large number of molecular actions not seen in atenolol. This is reflected in differential gene expression both in well-oxygenated and ischemic preparations. **J Clin Basic Cardiol 2008; 11 (online): 16–23.**

Key words: *ischemia, betablocker, myocardium, gene expression*

Ischemic heart disease is still the main cause of death in the western hemisphere despite considerable improvement of primary and secondary prevention, diagnostic techniques and treatment during recent years. Lack of understanding of the molecular mechanisms of anti-ischemic cardioprotection and dysfunctional metabolism contribute largely to cardiovascular disease morbidity and mortality. Ample epidemiologic and clinical evidence suggests that cardiovascular disease will remain the number one threat to human health in the decades to come. Cardiovascular risk factors have been investigated on a large scale. Primary as well as secondary prevention has improved cardiovascular morbidity and mortality in recent years and both involve the intensive use of betablockers [1–14]. In this context, comparatively little is known about molecular mechanisms of CHD and myocardial tolerance towards ischemia. However, the topic has recently received considerable attention. The investigation of multiple gene expression pattern during ischemia, hence, appears to be of high priority in fighting coronary death [15–21].

Administered before, during and after myocardial ischemia, betablockers support the preservation of myocardial integrity [22–33]. Nebivolol constitutes a new type of betablocker, which shows properties different from those of other betablockers [23–27, 30–32]. It exerts various additional effects, such as acting on the endothelial NO-pathway and an extremely high affinity to the β1-adrenoceptor. It also shows a high affinity to serotonin receptors of the 5-HT$_{1A}$ subtype and has been attributed a considerable anti-arrhythmic [29–34] and cardioprotective effect.

Since nebivolol shows numerous pharmacological properties which may entail anti-ischemic protection, we analysed the molecular signature by quantitative assessment of the expression of approximately 23,000 genes in human atrial myocardium under ischemic conditions in the presence and absence of nebivolol and compared its effect to that of atenolol. Measurements by our group indicate numerous pathways and biological processes differentially expressed when experimental ischemia was performed in the presence of nebivolol [35]. We found that, in the presence of nebivolol, processes that are mainly involved in myocardial ischemia, such as 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 [35]. From these data one can conclude that nebivolol may exert an important cardioprotective action on a variety of intracellular pathways. The present work, however, is intended to study the differences in expression profiles between nebivolol and a standard betablocker, atenolol, in hypoxia and normoxia.

Little is known about the molecular effects of nebivolol upon myocardial ischemia. In our earlier work, 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 demon-

strates the activation of repair mechanisms and changes in the metabolic state of the cell. We studied myocardial gene expression profiles in the presence and absence of nebivolol and compared these with the results of the same type of experiments retrieved in the presence of atenolol. The strength of the study lies in the fact that human myocardium is used and results can be well interpreted for patients with ischemic heart disease.

While a number of authors indicate that in experimental/ clinical myocardial ischemia gene expression is altered, gene expression profiling in ischemia has not been looked at extensively in human myocardial tissue under betablockade so far [35, 36]. Hence, it remains unknown whether or not the effect of specific betablockers can be related to an altered pattern of gene expression during myocardial ischemia. The latter, in form of a particular molecular signature, could reveal a new understanding of cardioprotective mechanisms. Using this technique, relevant cardio-protective properties of anti-ischemic drugs could be more easily assessed. In order to gain more understanding of the relevant molecular pathways and biochemical processes involved, the effect of betablockers should be looked at in this project.

Methods

Methods have been well established by our group over the years in the course of various other projects and in co-operation with Core Facilities Molecular Biology at the Center of Medical Research (ZMF) of our University [15, 20, 35–40]. All co-workers involved in the ongoing projects are well-experienced. Preliminary experiments in the planning phase of the project have already been performed at the ZMF.

1. Myocardial tissue samples were derived from the right auricle of patients undergoing cardiac surgery. A small part of the right auricle was removed when the heart was put on extracorporeal circulation and was normally wasted. The muscle piece was then placed in cooled Tyrode solution and transported to the laboratory where it was placed into the experimental chamber as done in earlier experiments [15, 35, 36]. The preparation was oxygenated and then snap-frozen. The model allowed to produce chemically and mechanically hypoxia/ischemia either by switching oxygen to nitrogen (hypoxia) or by using deoxiglucose instead of glucose, both or by immersing the preparation in a layer of paraffin oil [18, 41]. Here, we used the hypoxia technique as described below.

2. Gene expression was assessed by microarray technique [40, 42–47]. The combined technique of working on a living human preparation in an experimental chamber and the application of microarray technique was established in our laboratory of Experimental Cardiology over a period of several years to enable us to answer the particular question of interactions of hypertension, ischemia and gene expression in the human heart [15, 35, 36, 42]. The myocardial strip was instantly placed in well-oxygenated, cooled Tyrode solution and transported to the laboratory where it was fixed in an experimental chamber. The preparation then was oxygenated (100 % O_2) before being cut in two pieces of about equal size. Each of the preparations was then put into another individual chamber as done in earlier experiments [15, 35, 36]. Experimental ischemia was brought about by switching 100 % oxygen to 100 % nitrogen (hypoxia) in one of the chambers [41]. After 30 minutes of simulated ischemia the tissue samples were snap-frozen using liquid nitrogen. By doing so, we were able to compare ischemic and non-ischemic tissues of the same patient. Snap-frozen samples were stored at –70 °C until RNA isolation. Preparation for RNA isolation was made by homogenisation of the paraffin-embedded samples using a cryostatic microtome (HM 560 CryoStar, Microm).

3. Solutions: the preparations are continuously perfused with Tyrode solution [18, 20] 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. Experiments were performed using a 0.002269 molar stem solution (0.23 g nebivolol + 250 ml aqua dest.) of both nebivolol and atenolol, whereof 0.5 ml were added to 50 ml tyrode, which resulted in a 22.47 micromolar experimental solution.

4. RNA isolation and cDNA transcription: total RNA was extracted using the TRIZOL® 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 was resuspended in approximately 30 µl TE buffer. Then quality as well as quantity of RNA were assessed using spectrophotometry [46]. The quality of the isolated RNA was also analysed using the Agilent's Bioanalyzer 2100 system. Either 20–40 µg or 0.2–1 µg total RNA, when only reduced amounts of material were available, was then directly or indirectly via in-vitro transcription transcribed into DIG-labelled cDNA [21, 40, 42, 47].

5. Microarray [15, 35, 43–47]: the labelled samples were hybridised onto the array for 16 hrs. Subsequently, arrays were washed and detection was carried out using alkaline-phosphatase-conjugated anti-DIG antibodies and the appropriate substrate according to a highly standardized protocol. Arrays were scanned with the AB1700 Chemiluminescence Array Reader and images, raw data and tissue information were 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 CDS only. About 4700 control spots are used to assess array performance in each single step from array fabrication to assay readout. 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 oligonucleotide added to the hybridisation mixture (Fig. 1). This novel approach is crucial for optimal localization of each spot and allows normalization strategies that are not necessarily dependent on a high-end bio-informatics support. In each experiment, we use pooled data of four experiments as well as one individual single experiment (n = 4 + 1).

6. Operator/s: the AB1700 microarray system available at the Core Facility Molecular Biology is operated by four 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 and that was performed at approved inter-
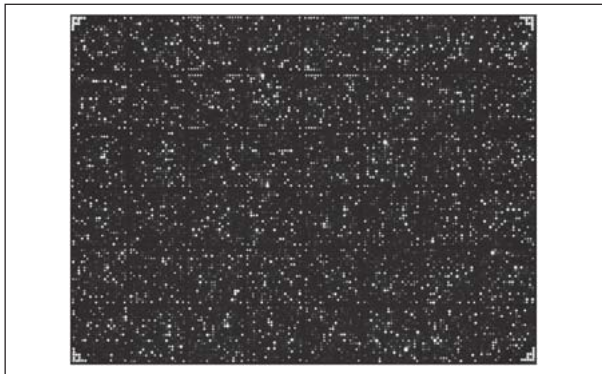
Figure 1. Typical results derived from microarray experiments. Light intensities on the microtiter plate reflect gene expression.
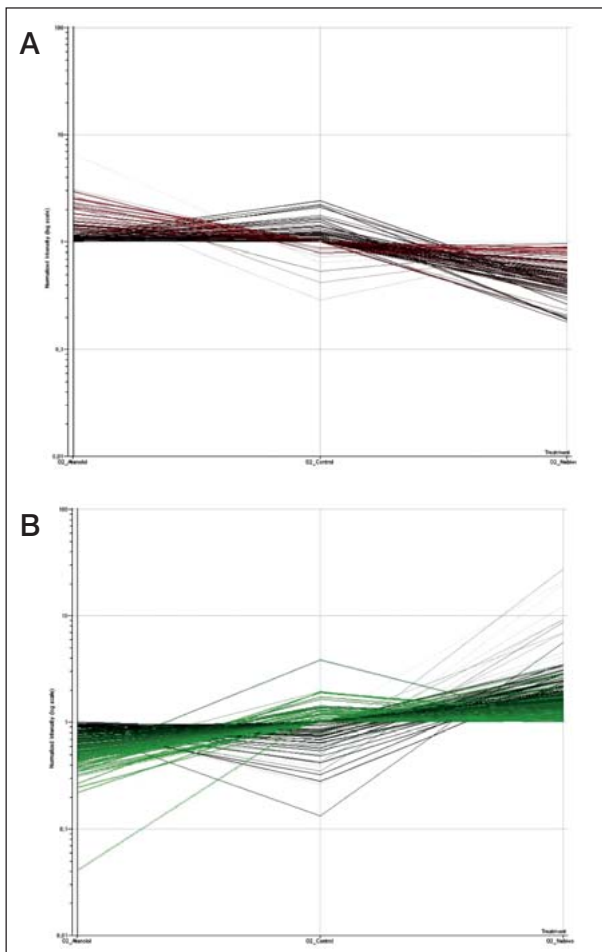
Figure 2. Panels A und B show all genes regulated by either atenolol or nebivolol under non-ischemic, well-oxygenated conditions. **Panel A** (red) summarizes all genes down-regulated by nebivolol compared to those up-regulated by atenolol during normoxia. Hence, panel A demonstrates the direct influence of atenolol and nebivolol on gene expression in well-oxygenated preparations. It can easily be seen that, under optimal conditions, without any sign of ischemia/hypoxia, the effect of nebivolol upon gene expression is quite different from that of atenolol. O_2 control indicates gene expression in well-oxygenated preparations without betablockers. O_2 nebivolol indicates gene expression in the presence of nebivolol in non-ischemic, well-oxygenated preparations. The red and green lines indicate the direction of gene regulation. On the left side of panel A, O_2 atenolol represents genes differentially expressed under atenolol during control conditions. Panel B, accordingly, shows all genes up-regulated by nebivolol which are down-regulated by atenolol (O_2 control here also representing untreated controls).
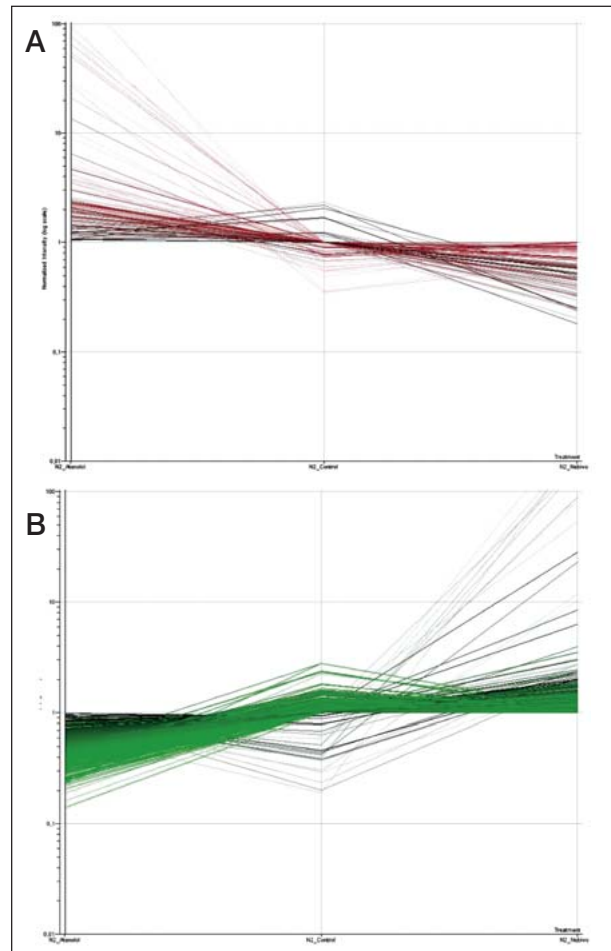
Figure 3. Panels A und B show all genes regulated significantly during experimental myocardial ischemia (hypoxia) in the presence and absence of either atenolol or nebivolol. **Panel A** (red) summarizes all genes down-regulated by nebivolol compared to those up-regulated by atenolol during hypoxia. N_2 control indicates gene expression in ischemic preparations without betablockers. N_2 nebivolol indicates gene expression in the presence of nebivolol in ischemic preparations. The red and green lines indicate the direction of gene regulation. On the left side of panel A, N_2 atenolol represents genes up-regulated under atenolol during hypoxia. **Panel B**, accordingly, shows all genes up-regulated by nebivolol which are down-regulated by atenolol (N_2 control here also representing non-treated controls).

national facilities. The novel AB1700 chemiluminescence microarray system turned out to be superior with respect to sensitivity and reliability.

Results

The present project is designed to study multiple gene expression under experimental ischemic conditions using an appropriate experimental chamber (see Methods). PANTHER software was used to obtain specific understanding of possible molecular pathways and biological processes involved in response to ischemia [35]. Here we elaborate the differences in gene expression profiles occurring between different types of betablockers such as new-generation nebivolol and older drugs like atenolol. We expect a variety of differences since they entail basic pharmacological differences [23–34].

We found evidence for differential gene expression in substrates with and without betablockade under ischemic conditions (see also [35]). Furthermore, we were able to demonstrate that the effects seen are not just class effects. On

the contrary, there is an enormous difference between gene expression profiles brought about by nebivolol and the standard betablocker atenolol both under ischemic and control conditions. Results were statistically evaluated in co-operation with the "Biostatistische Beratung ZMF" of the Medical University Graz using PANTHER software. Here we show the results of a microarray study of biological processes and pathways regulated by nebivolol but not by atenolol in well-oxygenated preparations and during experimental ischemia (testing nebivolol versus atenolol under simulated myocardial ischemia).

The panels below illustrate the impressive multitude of differentially expressed gene profiles between atenolol and nebivolol. One can see a pronounced difference between nebivolol and atenolol in gene regulation after 30 minutes of simulated ischemia. The following diagrams express biological processes and pathways differentially regulated during ischemia by nebivolol and atenolol. Diagrams, in fact, show processes and pathways which are significantly regulated under nebivolol but not under atenolol.

Experiments demonstrate that betablockers do not exert specific class effects on gene expression profiles in human myocardium under both well-oxygenated and hypoxic conditions. Rather, each of the investigated drugs shows an individual pattern of subsequent gene expressions. One may deduce that class effects are limited to the direct effect on the receptor, but a majority of differential effects lies beyond the direct receptor-mediated signal and unfolds likely time- and concentration-dependently by directly or indirectly modulating molecular pathways or biological processes. Hence we find very different expression profiles under atenolol and nebivolol in well-oxygenated and hypoxic preparations (Figs. 2, 3). Using the PAN-THER software we analysed the results derived from measuring over 23,000 genes expressed and found a number of biological processes and molecular pathways differentially regulated. For example, Figure 4 shows biological processes more than twofold down-regulated in the presence of nebivolol but unaffected by atenolol during simulated ischemia (100 % O_2 replaced by 100 % N_2 perfusion). One can see that, in particular on the level of reverse transcription and T-cell-mediated immunity as well as in the process of angiogenesis, a noteworthy regulation is brought about by nebivolol. Similarly, as in Figure 4, biological processes involved in cell-mediated immunity and reverse transcription are significantly up-regulated by nebivolol but not by atenolol. One may deduce that both cellular immunity as well as reverse transcription are regulated by nebivolol at various sites. In well-oxygenized preparations, we can see that numerous biochemical processes, mainly those involved in signalling and cellular immunity, are affected by nebivolol but not by atenolol (Figs. 5–7). In Table 2 (= Fig. 7), we can see that many processes involved in contraction, lipid metabolism, and proliferation are down-regulated by nebivolol only (not by atenolol). This kind of processes, if slowed down, may render the heart less vulnerable to O_2 deficit or ischemia. In well-oxygenated preparations, we can see that numerous biochemical processes are up-regulated by nebivolol (not by atenolol; Figs. 8, 9). Reverse transcription, stress response, and protein phosphorylation are of interest. We also find that nebivolol, but not atenolol, leads to a down-regulation of gene expression in glutamine-glutamate conversion and pyruvate metabolism, the latter of particular interest in ischemia (Fig. 10). Figures 11 and 12 show that, in well-oxygenated prepara-
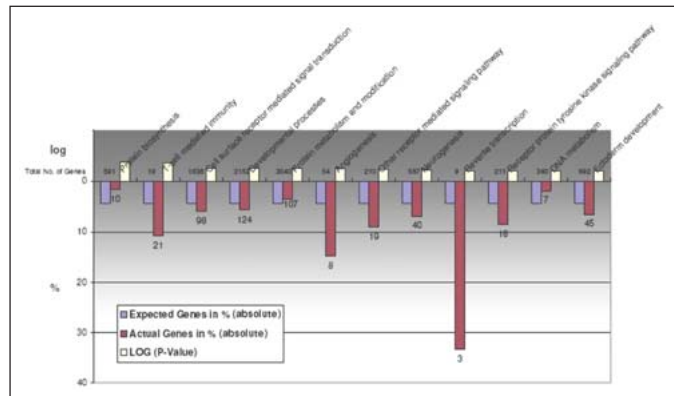
Figure 4. This figure shows biological processes more than twofold down-regulated in the presence of nebivolol but unaffected by atenolol during simulated ischemia (100 % O_2 replaced by 100 % N_2 perfusion). One can see that, in particular on the level of reverse transcription and T-cell-mediated immunity as well as in the process of angiogenesis, a noteworthy regulation is brought about by nebivolol.
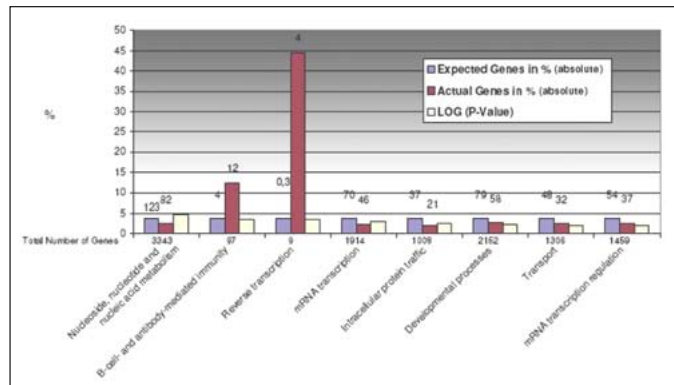

Figure 5. Similarly, as in Figure 4, biological processes involved in cell-mediated immunity and reverse transcription are significantly up-regulated by nebivolol and not by atenolol. One may deduce that both cellular immunity as well as reverse transcription are regulated by nebivolol at various sites.

tions, one can again see that numerous biological pathways, mainly those involved in signalling, angiogenesis, cellular immunity, and EGF generation, are affected by nebivolol but not by atenolol. Concerning nebivolol-regulated biological pathways, we could see that, during normoxia, up-regulation of pathways is hardly pronounced by nebivolol over atenolol. This becomes evident also in Figure 13, only 3 pathways are affected here. The interleukin signalling pathway is noteworthy, too.

The study will help differentiate between class effects of betablockers and specific effects brought about by nebivolol alone. So far, we have learned from these results that ischemic myocardial tissue from patients with and without betablockade shows altered patterns of gene expression profiles and that the latter largely depend on the betablocker used.

Discussion

Quality as well as quantity of isolated mRNA were double-checked using both spectrophotometry as well as an Agilent's Bioanalyzer 2100 system. Negative controls showed no activity or contamination. Microarray technique is a well-established method in the Core Facility Molecular Biology (CF-MB) of our University and was operated and supervised by an experienced team consisting of four members with many years of experience in various microarray techniques and platforms (Affymetrix, cDNA and oligonucleotide arrays)
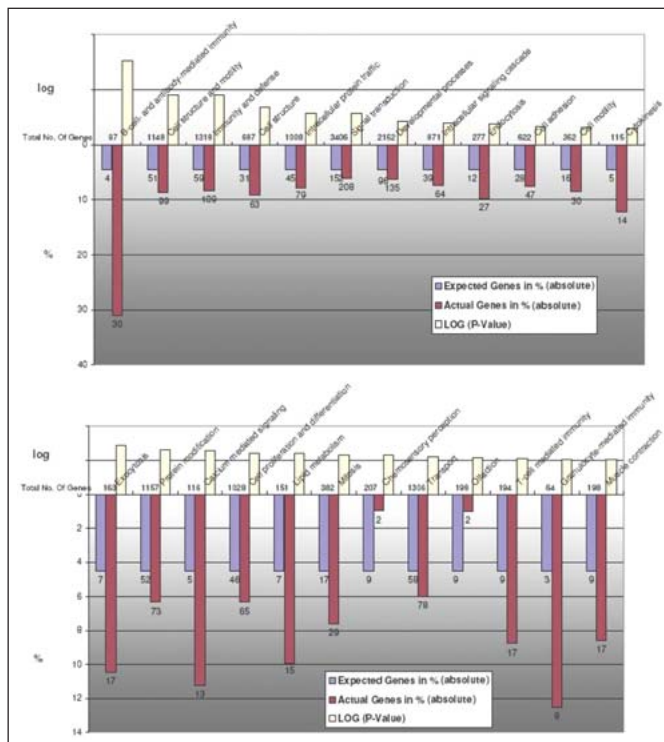
Figures 6 and 7. In well-oxygenized preparations, we can see that numerous biochemical processes, mainly those involved in signalling and cellular immunity, are affected by nebivolol but not by atenolol. In Figure 7, we can see that many processes involved in contraction, lipid metabolism, and proliferation are down-regulated by nebivolol only (not by atenolol). This kind of processes, if slowed down, may render the heart less vulnerable to O$_2$ deficit or ischemia.
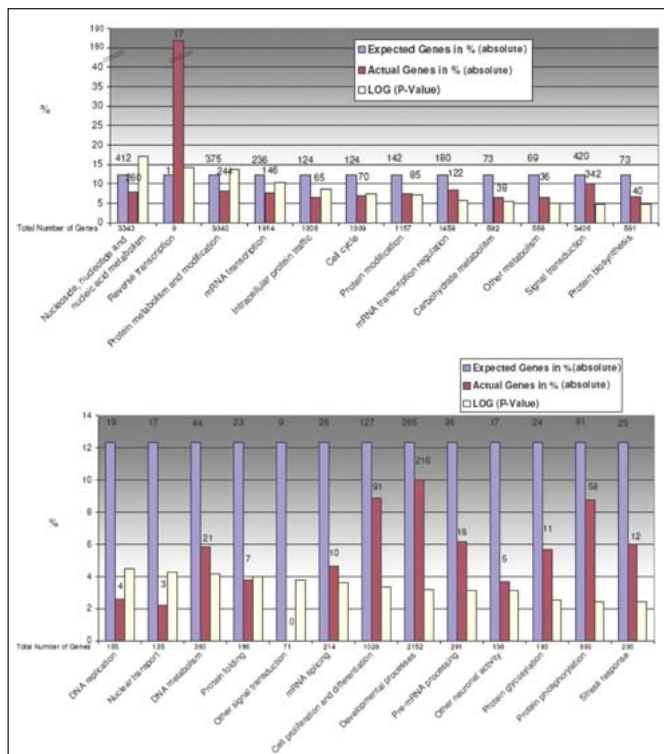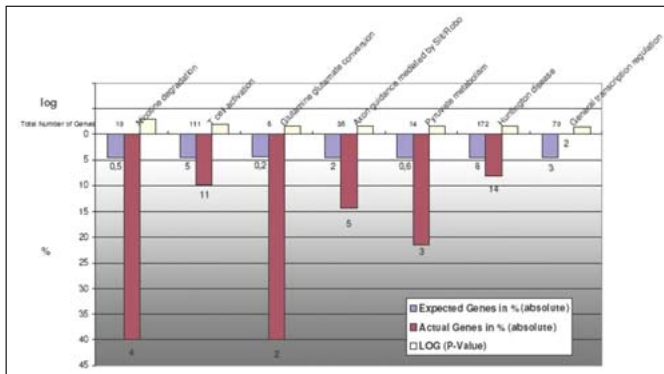

Figures 8 and 9. In well-oxygenized preparations, we can see that numerous biochemical processes are up-regulated by nebivolol (not by atenolol). Interesting are reverse transcription, stress response, and protein phosphorylation.

[15, 35, 36]. To evaluate the performance of the Applied Biosystems microarray technology a multi-center proof-of-principle study was conducted by the CF-MB (ZMF), which included Affymetrix, cDNA- and oligonucleotide-based platforms and that was performed at approved international facilities. The AB1700 chemoluminescence microarray system turned out to be excellent with respect to sensitivity and reliability. The results of our experiments are reproducible and experiments were conducted in accordance with international standards [42–47].

Since nebivolol shows numerous pharmacological properties which may entail anti-ischemic protection, we analysed the molecular signature by quantitative assessment of the expression of 23,000 genes in human atrial myocardium under ischemic conditions in the presence and absence of nebivolol and compared its effect to that of atenolol. Preliminary measurements [35, 36] by our group indicate numerous pathways and biological processes differentially expressed when experimental ischemia is performed in the presence of nebivolol. We saw that, in the presence of nebivolol alone, 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 we concluded that nebivolol may exert an important cardio-protective action on a variety of intracellular pathways. Our data confirm those by other authors attributing cardio-protective effects to nebivolol [48, 49]. Hence, in the present work, we intended to study the differences in expression profiles between nebivolol and a standard betablocker (atenolol) during hypoxia and normoxia.

The results of our present study show that, in particular on the level of reverse transcription and T-cell-mediated immunity as well as in the process of angiogenesis, a noteworthy regulation is brought about by nebivolol. Both are cellular key functions in the context of ischemia and regeneration. Similarly, biological processes involved in cell-mediated immunity and reverse transcription are significantly up-regulated by nebivolol but not by atenolol. Cell-mediated immunity is largely involved in tissue repair. One may deduce that both cellular immunity as well as reverse transcription are regulated by nebivolol at various sites. In well-oxygenized preparations, we showed that numerous biochemical processes, mainly those involved in signalling and cellular immunity, are affected by nebivolol but not by atenolol. This shows that, in non-ischemic, well-perfused preparations, nebivolol unfolds particular, possibly cardioprotective effects which cannot be observed under atenolol. Similarly, we can see that many processes involved in contraction, lipid metabolism, and proliferation are down-regulated by nebivolol only (not by atenolol). This kind of processes, if slowed down, may render the heart less vulnerable to O$_2$ deficit or ischemia. In well-oxygenized preparations, we found numerous biochemical processes up-regulated by nebivolol (not by atenolol): of special interest in this context are reverse transcription, stress response, and protein phosphorylation. It is interesting that nebivolol but not atenolol leads to a down-regulation of gene expression in glutamine-glutamate conversion and pyruvate metabolism, the latter of particular interest in ischemia.

Figure 10. It is interesting that nebivolol but not atenolol leads to a down-regulation of gene expression in glutamine-glutamate conversion and pyruvate metabolism, the latter of particular interest in ischemia.
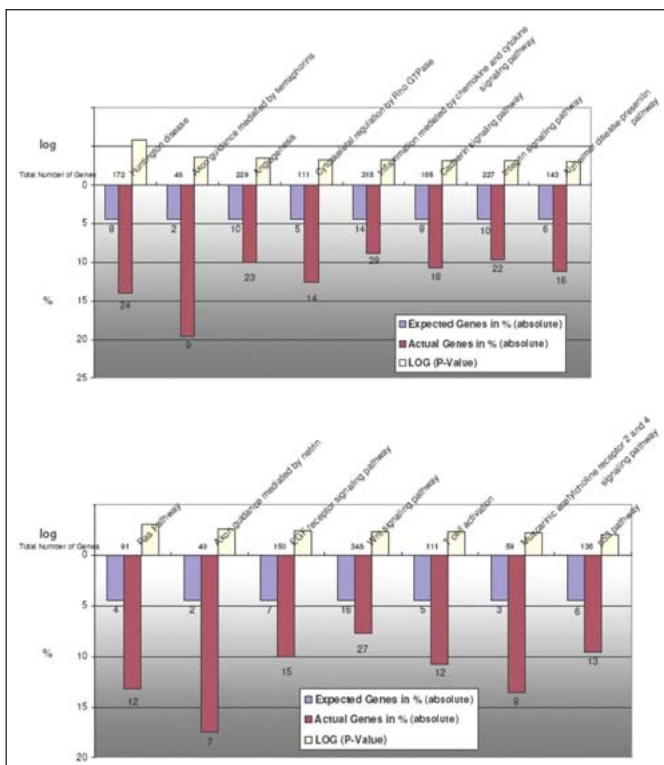
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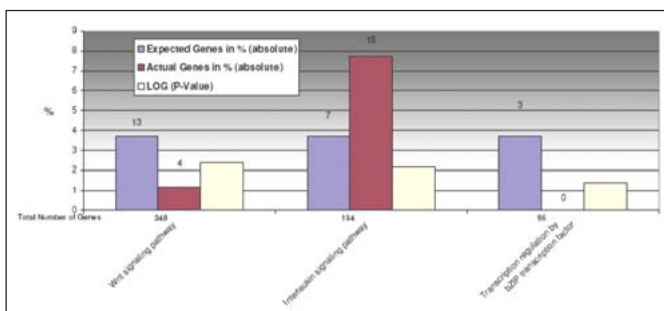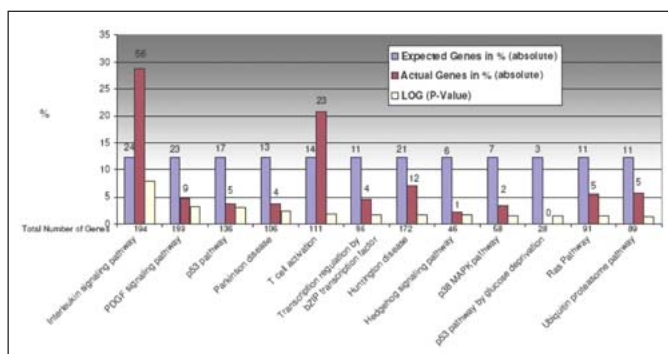

Figure 13. Generally, we could see that, during experimental ischemia, up-regulation of pathways is minimal by nebivolol over atenolol. This becomes evident in Figure 11, only 3 pathways are affected. However, the interleukin signalling pathway may be of interest in connection with inflammation, scarring and remodelling.

Generally, we saw that, during experimental ischemia, up-regulation of pathways is minimal by nebivolol over atenolol. This becomes evident in Figure 11, only 3 pathways are affected. However, the interleukin signalling pathway may be of interest in connection with inflammation, scarring, and remodelling. In well-oxygenized preparations, we can see that numerous biological pathways, mainly those involved in signalling, angiogenesis, cellular immunity, and EGF are affected by nebivolol but not by atenolol. Concerning nebivolol-regulated biological pathways, we saw that, during normoxia, up-regulation of pathways is hardly pronounced by nebivolol over atenolol. From these data we can certainly deduce that nebivolol unfolds a large number of molecular actions not seen in atenolol. This is reflected in differential gene expression both in well-oxygenated and ischemic preparations.

We also looked at special genes particularly regulated by nebivolol and not by atenolol during simulated myocardial ischemia, which could be of broader interest in the context of anti-ischemic protection. One regulation site of possibly major interest could be DDAH (dimethylarginine dimethylaminohydrolase) [50, 51] expression decreased in the presence of nebivolol. Methylation of arginine in proteins and subsequent proteolysis sets free methylarginines, in particular asymmetric ADMA, an inhibitor of nitric oxide synthetases (NOS). ADMA is transformed by dimethyaminohydrolase (DDAH) to citrulline (CIT) and dimethylamine (MA). ADMA is recognised as a plasma marker of increased cardiovascular risk but it is unclear whether it ever accumulates to sufficient levels to affect NO pathways. However, it has been shown that loss of DDAH function leads to a rise of plasma and tissue ADMA levels. On the other hand, it is likely that a feedback mechanism exists which regulates DDAH expression upon the availability of NO. In this context, it has to be mentioned that nebivolol can stimulate an increase of endothelial NO which becomes available at the vascular smooth muscle and induces vasorelaxation. Nebivolol seems to interact with the endothelial NO pathway in two complementary ways: it increases NOS activity and reduces the NO-scavenging radical superoxide anion by re-directing deranged NOS activity. Nebivolol appears to prevent the pathological ROS-induced depression of intracellular NO levels. The measured decrease of DDAH seen under nebivolol but not with atenolol could be a measure for the increased availability of NO brought about by nebivolol as a feed back control. This is of interest since several steps in the pathways of interaction have remained unclear. In the present study, we found that the expression of DDAH is reduced in the presence of nebivolol in both normoxia as well as hypoxia. It is certainly promising to investigate further into this interrelation of NO, DDAH, and nebivolol.

From our experiments, we learn about myocardial gene expression both in ischemic heart disease and under cardioprotective therapy. We can see large differences in expression profiles between nebivolol and atenolol, clearly indicating major differences in action sites. In the long run, our results may also provide new approaches in primary prevention as well as for direct myocardial tissue protection in ischemic heart disease by further justifying and prompting the development of highly specific pharmacological variants of betablockers. Further experiments will be needed to verify

Figure 14. Concerning nebivolol-regulated biological pathways, we could see that, during normoxia, up-regulation of pathways is hardly pronounced by nebivolol over atenolol. This becomes evident also in Figure 11, only 3 pathways are affected here. Noteworthy here, too, the interleukin signalling pathway.

expression of the most important genes differentially expressed under atenolol and nebivolol during ischemia such as DDAH, PDK, NCX, FGF, and AGAT by Real-time PCR.

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