Summary of Doctoral (Ph.D.) Thesis

EXAMINATION OF THE VASOMOTOR FUNCTION OF CORONARY MICROVESSELS IN IN PATIENTS WITH METABOLIC SYNDROME

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"With an excess of fat diabetes begins,
and from an excess of fat diabetics die…"
EP Joslin, 1927

1. INTRODUCTION

Obesity or weight gain of pathologic grade is already regarded as a pandemy in recent days, which is spreading throughout the whole world including more and more well-developed countries. 1.7 billion people suffer from obesity worldwide, and the prevalence of obesity demonstrates a continous increase. It is widespread even among young adults and children nowadays. Its prevalence rose from 44.8 % to 65.2 % in the adult population of the United States during the past four decades. To determine and characterize the grade of obesity body mass index (BMI) is a useful tool, which is determined by the quotient of the body weight expressed in kilograms and the square of the height expressed in meters. An adult person can be regarded as having overweight with a BMI between 25-29.9, while one is considered to be obese with a BMI over 30. It is a well-known fact that the risk of developing type 2 diabetes mellitus rises exponentially with the increase of BMI. Similar correlation can be detected between BMI and cardiovascular mortality. The metabolic syndrome, an entity involving metabolic changes and cardiovascular risk factors, plays a significant role in the development of cardiovascular diseases, with regard to the fact that the rate of cardiovascular mortality increases in proportion to the number of risk factors present. Metabolic syndrome has a prominent role with regard to cardiovascular diseases. According to the definition included in the commitment of the metabolic working group of the Hungarian Diabetes Association published in 2002, metabolic syndrome is an endemic and being in association with the civilization process. It is a metabolic disturbance showing few symptoms and developing in a latent fashion, its development is due to genetic

predisposition and inadequate lifestyle and nutrition. Metabolic syndrome causes atherosclerotic changes and is accompanied by early cardiovascular mortality. Insulin resistance stands in the background of the process, insulin resistance may be accompanied by hyperinsulinaemia, hypertension, central obesity, atherogenic dyslipidaemia and impaired glucose tolerance, however, it may coexist with other metabolic and hemostatic disturbances. Certain shift of emphasis has been presented among the components of the syndrome recently, and central obesity has come into focus. According to the latest definition created by the International Diabetes Federation, diagnosis of the syndrome can be established if at least of the above mentioned metabolic changes are present beyond central obesity. The presence of central obesity can be established when having an abdominal circumference equal to or over 94 centimeter among European men and 80 centimeter among European women. The probability of development of a new diabetes rises to about 3,51-fold in the presence of having metabolic syndrome. Based on the results of another clinical trial, cardiovascular mortality rates rise to 2,07-fold in case of having metabolic syndrome, 3,53-fold in case of having diabetes mellitus and 8,19-fold in the coexistance of both diseases. Here we mention Haffner's ticking clock theory, according to which the clock starts ticking at the time of development of insulin resistance. It starts ticking faster when elevated blood glucose levels occur, and it stops ticking even faster when manifested diabetes mellitus has developed. At the time of establishing the diagnosis of type 2 diabetes mellitus more than 50 % of the patients suffer from a manifested cardiovascular disease and more than 80 % of these diabetic patients die of a cardiovascular cause. Based on epidemiologic studies data the presence of type 2 diabetes mellitus developing on the grounds of a metabolic syndrome carries a 2 to 4-fold increase of risk in the development of different cardiovascular events. There are staggering statistical data proving that the risk of development of myocardial infarction in diabetes mellitus is

identical with the increase of risk in patients who already got over a myocardial infarction previously due to any other etiology.

It is well-known that a so-called accelerated atherosclerosis envolves in the coronary macrovessels as a consequence of a long-standing human metabolic syndrome and the consecutive type 2 diabetes mellitus, and this accelerated atherosclerosis largely contributes to the increase of cardiovascular mortality. Previous trials demonstrated that prior to and in parallel with the atherosclerotic changes concerning the large epicardial coronary branches the functioning of the coronary microvasculature is damaged in metabolic syndrome. This dysfunction may lead to alterations in cardiac tissue perfusion even prior to the occlusion of the main coronary branches and, ultimately to ischaemic myocardial injury. The exact nature of coronary microvessel functioning in patients suffering from a metabolic syndrome and the definite pathophysiologic mechanisms standing in its background are not sufficiently known.

2. OBJECTIVES

Metabolic syndrome has a multifactorial etiology and the major pathologic conditions characterizing it such as pathologic grade obesity, hypertension and diabetes mellitus may jointly affect the functioning of the cardiovascular system. The mechanisms how individual pathologic conditions existing in metabolic syndrome alter the vasomotor function of coronary vessels are not sufficiently clarified. Based on all of these statements I set out the following objectives in my scientific research:

1. To examine the vasomotor function of isolated coronary microvessels gained from patients having had cardiac surgery.

2. To clarify how the presence of individual pathologic conditions in metabolic syndrome such as obesity, hypertension and diabetes mellitus affect the vasomotor function of coronary microvessels

3. Furthermore, to examine the mechanisms mediated by endothelial and smooth muscle cells standing in the background of the dysfunction.

The results of our experiments and conclusions deduced from these results may assist in the understanding of the pathophysiological processes of coronary microvessel dysfunction developing in human metabolic syndrome. Moreover, they may contribute to more precise elaboration of pharmacological therapeutic principles, which are aimed to counterbalance altered microvessel function and to decrease cardiac risk probably developing due to microvessel dysfunction.

3. METHODS

3.1. Patients characteristics

All protocols were approved by the Ethical Committee at the University of Debrecen, Medical and Health Science Center. All patients were given written information about the experimental use of human specimen. Assessment of coronary arteriolar responses was performed in patients (N=38) who underwent cardiac surgery. 38 patients having had cardiac surgery previously were enrolled into the first phase of our experiments. The patients were divided into groups based on the presence (HT) or lack (non-HT) of documented hypertension. Further patient subgroups were established during the further analyses based on the grade of obesity both in the normotensive and the hypertensive patient groups (normal body weight: body mass index BMI< 25, and obesity: BMI>30). In our other examinations concerning a separate group of patients (25 patients)

we performed patient division based on the distinction of whether they had diabetes mellitus previously or not, the latter had been cleared up by the medical history and patient documentations.

3.2. Isolated human coronary microvessel technique

We performed our experiments on isolated microvessels using coronary arterioles (about 100 μm in diameter). The coronary arterioles were isolated from a segment of the right atrial appendage removed either during coronary artery bypass surgery or heart valve replacement surgery. The right atrial appendage was fixed with needles in a silicone-based Petri dish containing cold (0-4 degrees Celsius, pH 7,4) Krebs solution (110,0 mmol/L NaCl, 5,0 mmol/L KCl, 2,5 mmol/L CaCl2, 1,0 mmol/L MgSO4, 1,0 mmol/L KH2PO4, 5,5 mmol/L glucose and 24,0 mmol/L NaHCO3). Then a 2-3-millimeter segment of the primary coronary arteriole was isolated using microsurgery tools and with the assistance of stereomicroscopy (Nikon SMZ 600). The isolated arteriole was first fixed by cannulating its one end (this was to become the proximal end), then blood cells were removed from the lumen by using a perfusion pressure of 20 Hgmm. Following that the distal end of the vessel was also cannulated, and the vessel was put into an organ bath system having constant temperature (T=37 degrees Celsius, pH=7,4), this process was done after the initial vessel length was preset with the assistance of a microscrew. A continous flow (40 ml/min) with oxygenated (O2: 10%, CO2: 5%, N2: 85%) Krebs solution was ensured in the organ chamber. The intraluminal pressure was raised slowly to 80 Hgmm with the assistance of a peristaltic pump (Living Systems Instrumentation, VT, USA) controlling pressure, and it was kept at 80 Hgmm for about 60 minutes until the condition of the vessel stabilized. The intraluminal pressure was continously measured with a pressure transducer throughout the whole period. Images were recorded with a digital camera (CFW1310, Scion Corp, USA)

attached to a microscope (Nikon, Eclipse 80i). The inner diameter of the isolated arteriole was measured using Image J software (NIH Image, MD, USA)

3.3. Examination of human coronary arteriole function using vasoactive agents

Spontaneous myogenic tone developed in the isolated coronary arteriole under the effect of applying 80 Hgmm of intraluminal pressure during the one-hour incubation period. We tested the responses of the isolated coronary microvessels in our experiments provoked by endothelium-dependent and endothelium-independent vasoactive substances, each having well-known mode of action. The applied vasoactive substances were administered in adequate target concentrations into the perfused bath tub with a known volume (15 mL) during the protocols. Applying cumulative concentrations the maximal effects of the adequate concentration of each individual substance impinged to the vessel diameter were continously recorded.

During the first series of experiments changes in diameter were measured provoked first by the endothelium-dependent vasodilator bradykinin (0,1 nmol/L- 1 μmol/L), then by sodium nitroprusside (SNP; 0,1 nmol/l – 1 μmol/L), an endothelium-independent vasodilator substance acting directly on smooth muscle cells. The individual cumulative dose-effect curves were followed by a wash-out period and an incubation period of 30 minutes, during which the arterioles regained their initial muscular tone.

Vascular responses provoked by an agonist (bradykinin, SNP) were investigated in certain protocols also in the presence of either indomethacine, a non-selective COX-inhibitor (10 μmol/L, 30 minutes incubation period), or NS-398, a selective COX2-inhibitor (10 μmol/L, 30 minutes incubation period). We made conclusions concerning the role of prostaglandin metabolites in the evolvement of agonist responses on the basis of these test results

3.4. Immunohistochemistry

Atrial appendages from DM(-) (n=8) and DM(+) patients (n=8) were embedded and frozen in OCT compound (Tissue Tek, Electron Microscopy Sciences). Acetone-fixed consecutive sections (10-μm thick) were immunolabeled with a polyclonal anti-COX-2 primary antibody (dilution 1:100, Cayman Chemicals). Immunostainings were visualized either by using avidin-biotin horseradish peroxidase visualization systems (Vectastain kit, Vector Laboratories), stained with DAB. Immunofluorescent labeling was performed with primary antibodies for COX-2 and smooth muscle α-actin (Novocastra) and with FITC or Texas Red labeled secondary antibodies (Vector Laboratories and Jackson Immuno Research, respectively). DAPI was used for nuclear staining. For nonspecific binding, the primary antibody was omitted. Images of the sections were collected with a digital camera (CFW 1310C, Scion Corp) connected to a Nikon Eclipse 80 microscope

3.5. Statistics

Data are expressed as means ± S.E.M. Agonist-induced arteriolar responses and myogenic tone were expressed as changes in arteriolar diameter as a percentage of the maximal dilation defined as the passive diameter of the vessel at 80-mmHg intraluminal pressure in a Ca2+-free medium, as described previously.

Data were stored and analyzed with the NCSS statistical software (Kaysville, Utah, USA). Test selection was based after evaluating the variables for normal distribution, employing the Kolmogorov-Smirnov test. Testing differences of different variables between normotensive and hypertensive groups was accomplished by two-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. One-way ANOVA followed by Tukey posthoc test was performed. Since all the obtained data in this study met the normality criteria accomplished by Kolmogorov-Smirnov test, associations

between continuous variables were analyzed by the Pearson correlation test. To examine categorical variables two-way ANOVA was performed with the categorical variable and hypertension, as the two factors. Those associations which were significant on univariate analysis were entered into a multiple regression model, adjusted for the significant covariates. In a linear regression analysis the slopes of regression lines were also compared between normotensive and hypertensive groups. Data are expressed as means ± S.E.M. $P < 0.05$ was considered statistically significant.

4. RESULTS

4.1. The effect of obesity and hypertension on vasomotor function of coronary microvessels

The effect of obesity and hypertension on vasomotor function were investigated in coronary arterioles isolated from patients (N=38) underwent coronary bypass or valve replacement surgery. Hypertensive patients exhibited higher actual blood pressure readings, which however did not reach statistical significance. In the two groups there was no difference in the body mass index (BMI) and the prevalence of other underlying diseases, medications as well as, the age, gender and the type of cardiac surgery. The endothelium-dependent vasodilator, bradykinin (0.1 nmol/L - 1 μmol/L) and the NO-donor, sodium nitroprusside (SNP, 1 nmol/L and 10 μmol/L) elicited substantial dilation in isolated coronary arterioles. On the average, there were no significant differences between bradykinin-induced and SNP-evoked dilations of coronary arterioles isolated from normotensive and hypertensive individuals. However, when the magnitudes of coronary dilations were segregated into lean (BMI<25) and obese (BMI>30) patients groups a marked difference could be revealed between normotensive and hypertensive patients. Bradykinin-induced dilations were significantly reduced in obese-normotensive patients, when compared to

lean-normotensive (P=0.013), and interestingly also when compared to those of obese-hypertensive patients (p=0.025) by two-way ANOVA. It should be noted, that ANOVA also revealed a significant interaction between hypertension and obesity (p=0.001). Moreover, we have found that the NO donor, SNP-induced dilations were significantly reduced in lean-hypertensive individuals, when compared to lean-normotensive (P=0.017) and importantly also when compared to those of obese-hypertensive patients (P=0.021). In this case, ANOVA also revealed a possible interaction between hypertension and obesity indicated by the small P-value (P=0.083). Collectively, these findings suggested that in obese and hypertensive patients agonist-induced dilations of coronary microvessels are even enhanced, rather than further reduced, as one would expect. To elucidate and further validate the possible role of overweight and obesity affecting coronary arteriolar responses the impact of BMI on the magnitude of dilations was also investigated by the Pearson correlation test. We have found a significant, positive correlation between bradykinin-induced dilations and BMI in the hypertensive group, whereas a negative correlation was found in those of normotensive patients. Similarly, a positive correlation was observed between SNP-induced dilations of coronary arterioles and BMI in hypertensive, but not in those of normotensive subjects. The slopes of regression lines were also compared between normotensive and hypertensive groups. Evaluating bradykinin-induced responses, significant difference was found between the slopes of the two regression lines (P=0.008). Similarly, when the NO donor, SNP-induced responses were analyzed the slopes between normotensive and hypertensive groups was also found to be significantly different (P=0.041). Moreover, no significant associations were found in any other variables investigated on bradykinin-induced responses (age: *P*=0.617; gender/male: *P*=0.341, diabetes mellitus: *P*=0.496, coronary artery disease: *P*=0.809, high cholesterol levels: *P*=0.418, β-blockers: *P*=0.477, ACE-inhibitors: *P*=0.269, Nitrates: *P*=0.640, Diuretics: *P*=0.670, Lipid lowering drugs: *P*=0.444), and also

on SNP-induced responses (age: *P*=0.406; gender/male: *P*=0.612, diabetes mellitus: *P*=0.147, coronary artery disease: *P*=0.793, high cholesterol levels: *P*=0.340, β-blockers: *P*=0.516, ACE-inhibitors: *P*=0.934, Nitrates: *P*=0.739, Diuretics: *P*=0.410, Lipid lowering drugs: p=0.115).

4.2. The effect of diabetes mellitus on vasomotor function of coronary microvessels

First the endothel dependent bradykinin (0.1 nmol/L - 1 μmol/L) elicited substantial dilation in coronary arterioles, which was significantly greater in DM(+) than DM(-) patients (Figure 1a), with -log[ED50] values of 8.46±0.18 and 7.77±0.22 (P<0.05), respectively. In both groups, dilations were similar in response to the NO-donor, sodium nitroprusside (SNP, (1 nmol/L - 10 μmol/L, with -log[ED_{50}] values of 7.3±0.28 and 7.23±0.34 (*P*=NS), respectively. There was no significant differences between the two investigated groups in comorbidities, medical treatment and in type of heart operation. By multivariate analysis, only the presence of diabetes, independent of other risk factors and co-morbidities, predicted the enhanced vasodilation to bradykinin. The underlying mechanisms are not completely understood. It is known, that behind the bradykinin induced coronary responses besides NO and EDHF there are vasodilating prostaglandins (prostacyclins). Later we investigated the role of prostaglandins in the bradykinin induced dilation. The same protocols were repeated in the presence of indomethacin (10 μmol/L, for 30 min), a non-specific inhibitor of the COX. In arterioles of DM(+) patients bradykinin-induced dilations were reduced to the control level by the non-selective COX inhibitor, indomethacin. It is known, that both COX 1 and COX-2 isoform have role in the synthesis of prostaglandins. While the bradykinin induced vasodilation wasn't influenced by selectív COX-2 inhibitor in non diabetic patients, it was significantly decreased in coronary microvessels in human diabetics. In our investigation imunohistochemical studies were also performed in order to detect alteration in the arteriolar expression of COX-2. Compared to non diabetic

patients (DM-) we have found a marked COX-2 immunostaining in coronary arterioles of diabetic patients (DM+), which was localized both to the endothelial and smooth muscle layers of arteriolar wall.

5. DISCUSSION

5.1 The effect of obesity and hypertension on vasomotor function of coronary microvessels

Although it has been suggested that obesity increases the risk for developing coronary heart disease the impact of obesity on coronary vasomotor function, especially in the presence of other diseases, such as hypertension, is poorly understood. There is a general agreement that in hypertension vascular dysfunction develops, which may contribute to the enhanced cardiac risk in these individuals. Vasomotor dysfunction is primarily characterized by reduced endothelium-dependent dilations both in large conduit vessels and resistance arteries. In subjects with hypertension a reduced brachial artery relaxation to hyperemic flow (FMD) has been demonstrated previously. In obesity, an early development of vascular dysfunction has also been described. For instance, in obese children with risk for atherosclerosis impaired FMD and NTG-induced relaxation of brachial arteries was reported recently. These observations suggested that both hypertension and obesity are associated with impaired conduit artery relaxations. Because of the close correlation between coronary arterial function and brachial artery relaxation we hypothesized that simultaneous presence of hypertension and obesity has detrimental effect on coronary arteriolar vasomotor function. In humans with hypertension and obesity the possible alterations in the vasomotor function of coronary arterioles have not yet been elucidated.

The aim of this study was to investigate the impact of hypertension and obesity on dilations of human coronary arterioles to endothelium-dependent and

–independent vasoactive agents, bradykinin and SNP with known mechanism of action. Thus, we studied the responses of coronary arterioles, isolated from the heart of patients who underwent cardiac surgery. In addition, we aimed to investigate the role of action mediated by the endothel and the smooth muscle cells.

In isolated coronary arterioles we have found no differences in the magnitude of bradykinin- and SNP-induced dilations between normotensives and hypertensive patients. Because there were no major differences between the two groups in other investigated variables it seemed that the presence of hypertension has no significant impact on coronary arteriolar dilations in this study population. However, when the magnitudes of coronary dilations were investigated in lean versus obese subjects marked differences could be revealed between the normotensive and hypertensive groups. We have found that in normotensive patients, obesity was associated with reduction of agonist-induced coronary dilations (both bradykinin-induced, endothelium-dependent and also SNP-evoked, endothelium-independent). Also, we observed that in lean patients presence of hypertension was associated with impairment of coronary dilations. These observations are in line with the large body of literature, suggesting detrimental effects of hypertension and obesity on vasomotor responses.

However, the important and new finding of this study that in the simultaneous presence of hypertension and obesity, coronary arteriolar dilations to bradykinin and the NO-donor, SNP were markedly enhanced and also dilations were positively correlated with BMI in these hypertensive patients. These findings may explain the lack of differences in the averaged data regarding coronary dilations between normotensive and hypertensive groups (Figure 1a and 1b), in which patients with various body weights were lumped together. Taken together, our present findings strongly suggest that in hypertensive patients overweight and obesity is in close association with enhanced/adaptive arterial dilations in the coronary microvessels.

The mechanisms responsible for the observed effect of obesity on vascular function of hypertensive patients are likely to be complex, and need more investigation. It is known, that bradykinin-induced coronary arteriolar dilations and FMD of the brachial artery are considered being partly dependent on endothelium-derived relaxing factors, such as NO, whereas SNP and NTG-induced dilations are depend on the responsiveness of vascular smooth muscle to NO. Because, both endothelium-dependent (NO agonist)- and -independent (NO donor)-induced coronary arteriolar and brachial artery dilations were enhanced in obese, hypertensive patients, it is likely that primarily the enhanced sensitivity of vascular smooth muscle cells to NO is responsible for the observed alterations. Nevertheless, the possible involvement of endothelial mechanisms cannot be completely excluded. Interestingly, recent studies, elucidating alterations in vasomotor function in animal models of obesity also demonstrated preserved or even enhanced coronary arterial dilations. A very recent study has reported that in patients with morbid obesity, rapid weight reduction is associated with reduction of NO synthesis. These investigations support our present findings that in certain conditions, obesity could activate adaptive vascular mechanisms, among others by increasing the sensitivity of vascular smooth muscle to NO, aiming to maintain/enhance vasodilatory function of arterial vessels. At this time, however, one can only speculate regarding the physiological and/or clinical relevance of the present observation. The hallmark of essential hypertension is known to be an increased total peripherial resistance. Conversely, any increase in body mass (muscular or adipose tissue) requires a higher cardiac output and expanded intravascular volume to meet the elevated metabolic requirements. An enhanced dilatory function of coronary arterioles may reflect increased coronary blood flow and metabolism caused by hyperdynamic circulation early in hypertension and obesity. Interestingly, in early studies it has been postulated that obesity may protect a given patient from the deleterious effect of hypertension by decreasing hypertensive target organ

damage. Taken together, the present study is the first to show a close association between obesity and the magnitude of dilations of coronary microvessels and peripheral conduit arteries of hypertensive patients. Obesity seems to activate intrinsic vascular mechanisms, such as increased NO sensitivity, implying an important functional adaptation of arterial vessels in the coronary and peripheral circulation to the simultaneous presence of obesity and hypertension.

5.2 The effect of diabetes mellitus on vasomotor function of coronary microvessels

Although clinical evidence clearly shows that coronary heart disease is the major cause of morbidity and mortality in patients with diabetes mellitus the underlying mechanisms are not completely understood. Much less is known about the alterations in the coronary microcirculation in diabetic patients. More than a decade ago Nitenberg et al have shown that despite the presence of angiographically normal coronary arteries and normal left ventricular systolic function the coronary flow reserve is reduced in diabetic patients. Given that, it has been suggested that in diabetes mellitus epicardial atherosclerosis may not be the primary cause resulting abnormalities in coronary flow reserve, but rather this is due to reduction in dilator capacity of coronary vessels. In this context, in animal models of diabetes mellitus reduced agonist- and flow-induced, endothelium-dependent dilations of coronary arterioles were shown. In humans with diabetes presence of reduced endothelium-dependent dilation of the large conductance arteries seems to be also well established. Much less is known regarding the effect of diabetes on endothelial function of microvessels, especially in the coronary circulation. In isolated coronary arterioles obtained from diabetic patients, Miura and his colleagues have found a reduced dilation in response to hypoxia, and proposed that impairment of ATP sensitive K+-channel activation may be the underlying mechanism for this alteration. Also, in the simultaneous presence of NO synthase and cyclooxygenase (COX)

inhibitors they have found that bradykinin-induced, endothelium-derived hyperpolarizing factor (EDHF)-mediated dilations were preserved in the diabetic coronary arterioles. It is known that - in addition to NO and EDHF - bradykinin-induced coronary arterial responses can be mediated by dilator prostaglandins, such as prostacyclin. Bradykinin-induced prostacyclin release in the coronary circulation seems to be especially important when other vasodilator pathways, such as NO-mediated mechanisms are inhibited. Prostaglandins play key roles in the vascular homeostasis, including antithrombotic, antiproliferative and vasodilator effects. Both in animals and in humans COX-2 is the predominant source of vascular prostacyclin synthesis. Given that, an enhanced COX-2-derived prostacyclin synthesis might have a particular importance under pathological conditions, when COX-2 expression is significantly increased. In a mouse model of diabetes mellitus recent studies have found that vascular COX-2 expression is markedly increased, contributing to the altered prostaglandin-mediated responses of large arteries and arterioles. On the basis of aforementioned it seemed logical to hypothesized that vascular expression of COX-2 is enhanced in human coronary arterioles in diabetes, which may modulate prostaglandin-mediated responses. Our results suggest that in coronary arterioles of patients with diabetes mellitus the bradykinin induced vasodilation is increased probably because the increased COX-2 expression may contribute to an enhanced synthesis/release of dilator prostaglandin - most likely prostacyclin- upon stimulation. In the present study pharmacological inhibitors of prostaglandin synthesis were used to reveal the role of prostaglandin in mediation of bradykinin-induced dilation of isolated coronary arterioles. We found that non-selective inhibition of COXs by indomethacin did not significantly affect bradykinin-induced arteriolar dilation in the non diabetic group, but it significantly reduced bradykinin-induced responses to the control level in coronary arterioles of diabetic patients. Furthermore, selective inhibition of COX-2 (NS-398) also reduced bradykinin-induced coronary dilations in the

diabetic group, but had no effect on responses of vessels of non diabetic group. Since the reduction induced by selective COX-2 inhibitor was same in the magnitude as induced by indomethacin, these findings suggest that in coronary arterioles of diabetic patients bradykinin elicits COX-2-derived dilator prostaglandin release, contributing to the greater bradykinin-induced dilations. In addition to the functional experiments immunohistochemical studies were performed in order to detect alteration in the arteriolar expression of COX-2. Compared to non diabetic patients we have found a marked COX-2 immunostaining in coronary arterioles of diabetic patients, which was localized both to the endothelial and smooth muscle layers of arteriolar wall. These findings are in accordance with our previous observations obtained in an animal model of diabetes mellitus and extend them to that of human diabetes. Collectively, our results suggest that in coronary arterioles of patients with diabetes mellitus increased COX-2 expression may contribute to an enhanced synthesis/release of dilator prostaglandin - most likely prostacyclin- upon stimulation. There is a vascular dysfunction in diabetes mellitus, caused by different reasons. Recently, investigators raise the hypothesis that during the development of diabetes mellitus adaptive mechanisms may compensate for the impaired vascular function. In addition, at the site of atherosclerotic plaques a specific involvement of the upregulated COX-2 enzyme and consequent increased prostacyclin production has been proposed, which may limit platelet aggregation and thrombus formation. Also, a specific role for COX-2-derived prostaglandins has been proposed in flow-induced adaptive vascular remodeling in an animal model of atherosclerosis. In line with these ideas, our present data suggest that in human coronary arterioles upregulation of COX-2 and bradykinin-induced release of dilator prostaglandins, despite the likely presence of disturbed microcirculation may serve as an adaptive mechanism aiming to reduce the potentially detrimental effects of diabetes on coronary blood flow to cardiac tissue. These findings have drawn a great attention to prostaglandins

produced by the vascular endothelium. Our present findings raise the hypothesis that pharmacological inhibition of the prostaglandin synthesis in the arteriolar wall by the inhibition of COX-2, may adversely affect coronary vasodilator responses in patients with diabetes mellitus. Taken together, in diabetics there is an increased COX-2 expression, augmented prostaglandin-mediated bradykinin-induced dilation in coronary arterioles, which may represent a compensatory mechanisms aiming to maintain an appropriate blood supply of the myocardium.

6. SUMMARY

The multifactorial origin metabolic syndrome and its characteristic conditions like obesity, hypertension and diabetes mellitus all together have an effect on the function of cardiovascular system. The underlying mechanism, how this conditions change the vasomotor function of coronary microvessels has not yet been fully elucidated. This prompted us to investigate the vasomotor function of coronary arterioles, isolated from the heart of patients who underwent cardiac surgery. In addition, we aimed to investigate the role of action of obesity, hypertension and diabetes mellitus on vasomotor function of coronary microvessels. The key, novel findigns of our studies are the followings: 1.) There is a decreased vasodilation of isolated coronary microvessels in normotensive patients who suffered from obesity. 2.) However, among hypertensives agonists induced isolated coronary microvessel dilations were significantly enhanced in obese patients, when compared to lean individuals. By this observations I suppose that obesity may lead to activation of adaptive vascular mechanisms to enhance the dilator function of coronary and peripheral arterial vessels in hypertensive patients. Other part of our investigation shows 3.) that in isolated coronary arterioles of diabetic patients bradykinin induces enhanced COX-2-derived prostaglandin-mediated dilation compared with non

diabetic patients. It shows that in diabetes mellitus increases COX-2 expression and dilator prostaglandin synthesis in coronary arterioles, which may serve to increase dilator capacity and maintain adequate perfusion of cardiac tissues.

In summary, our present data suggest that in metabolic syndrome the different conditions, like obesity, hypertension or diabetes mellitus changes the vaomotor function of coronary microvessels on different ways. Despite, that probably all of this conditions damage the function of coronary microvessels, it can't be out of account, that there are some adaptív mechanisms, which could play role in maintenance of adequate perfusion of cardiac tissues in pathologic conditions.

LIST OF PUBLICATIONS:

***In extenso* publications related to the thesis**

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