SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

ASSESSMENT OF MICROVASCULAR FUNCTION IN ANIMAL MODELS OF OBESITY

by Dr. Attila Fehér

Supervisor: Dr. Zsolt Bagi

UNIVERSITY OF DEBRECEN
KÁLMÁN LAKI DOCTORAL SCHOOL OF THROMBOSIS, HEMOSTATIS, AND VASCULAR DISEASES

DEBRECEN, 2011

Assessment of microvascular function in animal models of obesity

By Dr. Attila Fehér, M.D.

Supervisor: Dr. Zsolt Bagi, Ph.D.

Kálmán Laki Doctoral School of Thrombosis, Hemostasis and Vascular Diseases, University of Debrecen

Head of the **Examination Committee**:	Prof. Dr. György Balla, MHAS
Members of the Examination Committee:	Prof. Dr. Tamás Bíró, Ph.D.
	Dr. Péter Hamar, Ph.D.

The Examination takes place at Department of Pediatrics, Medical and Health Science Center, University of Debrecen
February 24, 2012. 11:00

Head of the **Defense Committee**:	Prof. Dr. György Balla, MHAS
Reviewers:	Dr. Ferenc Domoki, Ph.D.
	Dr. Norbert Szentandrássy, Ph.D.
Members of the Defense Committee:	Prof. Dr. Tamás Bíró, Ph.D.
	Dr. Péter Hamar, Ph.D.

The Ph.D. Defense takes place at the Lecture Hall of the 1st Department of Medicine, Institute for Internal Medicine, Medical and Health Science Center, University of Debrecen
February 24, 2012. 13:00

1. INTRODUCTION

Obesity is a change in metabolism, where adipose tissue is accumulated in the human body to such an extent that can lead to several disease states, and is associated with decreased life expectancy and increased mortality. For modern societies, obesity is one of the greatest challenges humanity has to face. All over the world 1.7 billion people are overweight, and the prevalence of obesity is continuing to rise, nowadays it is not unusual to be observed among young adults and children. It is well known that obesity is associated with increased morbidity and mortality, and an increased risk for developing hypertension and coronary artery disease. It is also widely accepted that long standing obesity can enhance the development of atherosclerosis, which can further increase cardiovascular mortality. Recent studies have shown that in obesity, microvascular alterations can be observed well before the atherosclerosis of large conduit arteries. Previous investigations provided evidence regarding impaired microvascular function in the presence of diabetes mellitus or hypertension. A paucity of information is available regarding how the microvascular function is modified in the presence of obesity, especially regarding the potential differences in the microcirculation of different tissue types.

Based on the aforementioned findings, the main aims for my investigations are:

1. To investigate the possible disturbances in the resistance vessel function in animal models of obesity, specifically focusing on the skeletal and coronary microvascular function.
2. To elucidate the possible underlying mechanisms responsible for the obesity induced microvascular alterations.

3. Furthermore, to assess the effect of pharmacological agents that can play a role in the treatment of the potential obesity associated microvascular dysfunction.

Our experiments, and their potential conclusions can help us to understand the obesity associated microvascular changes and to reveal the underlying pathophysiological mechanisms. Fully understanding the alterations of resistance vessels can help us to develop specially targeted therapies, that can contribute to the treatment or prevention of the obesity associated comorbid diseases.

2. LITERATURE REVIEW

2.1 The regulatory role of the endothelium and smooth muscle cells under physiological conditions

The vascular endothelium is not just a barrier inside the vessel wall, but controls coagulation mechanisms, leukocyte and platelet adhesion, and last but not least, regulates local blood flow to maintain tissue perfusion. In response to several physiological stimuli the endothelium synthetizes and releases several vasodilatory and constrictatory agents, that can modify tissue perfusion through their effect on the vascular smooth muscle cells. In certain conditions the disruption of the fine balance between the endothelium and the vascular smooth muscle cells can lead to pathological function of the microvessels, so called microvascular dysfunction. In the presence of microvascular dysfunction a homeostatic imbalance between the vasodilators (nitrogen monoxide, prostacyclin, endothelium derived hyperpolarizing factor) and the vasoconstrictors (angiotensin 2, endothelin-1, vasoconstrictor prostaglandins) occurs, which can lead to the narrowing of the vessel luman and consequent reduction in tissue perfusion.

2.2 Peripheral microvascular function in obesity

Human obesity is often associated with comorbid diseases (e.g. high blood pressure) that can cause critical alterations in the microvascular function. It is widely accepted, that long standing hypertension can lead to vascular dysfunction, which is greatly responsible for the rise in cardiovascular risk. There is considerbale evidence demonstrating that obesity can modify endothelium mediated vasomotor function without the signs of hypertension. The animal models of obesity are great tools for

studying pathological changes that are otherwise not accessible in human subjects. There is a huge amount of experimental evidence showing that the function of the peripheral microvessels can be impaired in experimental obesity, however the underlying mechanisms and the precipitating factors are still not completely understood.

2.3 Coronary microvascular function in obesity

In order to be able to cardiovascularly match the weight gain associated increased metabolical requirements the body must icrease its cardiac output and its intravascular blood volume. It is well known, that obesity is often associated with left ventricle hypertrophy, that can be the first step in the process of accomodation to the increased hemodynamic and metabolic demands. The coronary vessels are also required to adapt to the higher demand, but the coronary circulation is unique in a way, that oxygen extraction happens at almost the maximal level, so a discrepancy between the metabolic demand and the tissue perfusion can have critical effect on the contractile function of the myocardium. The adaptation of the coronary blood flow to the altered metabolic demands is achieved by regulation of the vessel resistance at different levels of the coronary circulation by the cooperation of several mechanisms (e.g. myogenic flow response, metabolic diameter regulation). The large coronary arteries represent very little resistance in the coronary circulation; but the resistance against blood flow rapidly increases as the vessel diameter decreases below 300 μm. Based on this it is very essential to directly study the coronary arterioles. Although study results obtained from large coronary vessels can suggest the impairment of coronary vascular function in obesity, in fact in the level of the microcirculation the vasomotor tone and the agonist induced responses can remain preserved, especially in the early stage of the disease development. This rises the

hypothesis, that the regulatory mechanisms in the coronary microvascular system can be protected against the potentially harmful effects associated with obesity observed in the peripheral microcirculation.

2.4 Altered Rho kinase function in obesity

Rho kinase is a serine/threonine kinase GTP-Rho-binding protein, that is primarly responsible for modulation of cellular shape and cellular movements through its affect on the cytoskeleton. The Rho kinase interacts with insulin receptor substrate and therefore it also participates in the insulin signalling pathways. Increased activity of the Rho kinase has been described in various animal models of type two diabetes mellitus and is responsible for the impaired vasodilator function of the microvessels isolated from these animals. As of now there is no evidence whether Rho kinase function is altered in obesity.

2.5 The vasoprotective effect of resveratrol

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a natural polyphenol of plant origin, that has been studied extensively because of its potential beneficial effect on life expectancy. Epidemiological studies have shown that the mediterranean diet (rich in resveratrol) is associated with decreased cardiovascular risk. The resveratrol has been proven to have wide variety of anti-atherogenic effects, to inhibit LDL oxidation and platelet aggregation, to regulate vascular smooth muscle cell proliferation and to modulate NO production. Resveratrol improves the health of high fat diet fed obese mice and increases their survival. Recent studies provide evidence, that resveratrol assures significant vasoprotective effects in aging rodents.

2.6 The role of caveolae and calcium activated potassium channels in experimental obesity

In the microcirculation, endothelium dependent but NO and prostacyclin independent vasodilator mechanisms are of great importance. Compared to the NO pathway the endothelium derived hyperpolarizing factor (EDHF) mediated dilation depends less on the oxidative stress, so it can participate in the compensation of the obesity associated impaired NO function. The smooth muscle cell large conductance calcium activated potassium (BKCa) channels among the other calcium activated potassium channels play pivotal roles in the EDHF response. In evoking the EDHF mediated dilatory responses it is essential to redistribute several vascular signalling pathways into the caveolae. Caveolae are small (50–100 nanometer) invaginations of the plasma membrane, that are assembled by the connection of several important structural and scaffolding protein, such as the caveolin-1. A recently reported cell culture study claimed that BKCa channels are located into caveolae inside bovine aortic endothelial cells, where the caveolin-1 exerts a negative regulatory role on BKCa channels connecting to them through their α subunit. It is well known that BKCa channels are primary expressed by vascular smooth muscle cells inside the vascular wall, where they become activated when detecting high local concentration of calcium (10-100 μM). More experiments need to be performed to provide evidence whether microvascular calcium activated potassium channel function is altered in obesity.

3. METHODS

All of our animal experimental protocols were approved by the Institutional Animal Ethical Committee. Before the experiments the animals underwent overnight fasting. Anesthesia was induced by intraperitoneal injection of sodium pentobarbital (50 mg/kg), and then the gracilis muscle was dissected. Animals were euthanized by additional injection of sodium pentobarbital (150 mg/kg), and then the heart was dissected.

3.1 Animal models of obesity

In the first of our experiments we used 12 week old male obese Zucker (OZ) rats and their age matched controls (lean Zucker, LZ). The OZ rats have the homozygous mutation of the leptin receptor and by the age of 12 weeks their body weight is significantly higher than the weight of normal phenotype LZ rats. In the second part of our experiments we induced obesity in 20 week old male ECR wild type ($Nrf2^{+/+}$) and Nrf2 knockout ($Nrf2^{-/-}$) mice by feeding them with high fat containing diet for 16 weeks. The Nrf2 transcription factor is responsible for the expression of several genes, among others genes for various antioxidant enzymes, so it plays a substantial role in the regulation of oxidative stress. Animals were kept either on standard diet, on high fat containing diet (high fat diet, HFD) or on a resveratrol supplemented high fat diet for 16 weeks. In our third set of experiments we induced obesity in male Wistar rats by feeding them with HFD for 10 weeks. Furthermore for the exploration of the obesity associated microvascular alterations we employed 12 to 14 week old caveolin-1 knockout mice.

3.2 Analitical measurements

We used plasma obtained from control and HFD rats to determine total cholesterol, glucose and insulin levels.

3.3 Isolated microvessel technique

We performed our isolated microvessel experiments on isolated coronary and gracilis arterioles (~120 μm). On the day of the experiment after opening the chest and excision of the heart, the tissue was placed into a Petri dish containing ice-cold Krebs solution. After the tissue was pinned, microvessels were isolated with the help of microsurgical instruments. After isolation, the arterioles were transferred to a cold, oxigenated Krebs containing organ chamber, where they were cannulated. The intraluminal pressure was gradually set to 80 mmHg with an intraluminal pressure servo control system. After one hour of incubation the arterioles developed spontaneous myogenic tone. Changes in the inner diameter were detected with a digital camera and analyzed with a computer.

3.4 Assessing arteriolar function with the help of vasoactive agents

In our experiments we detected several vasoactive agent induced microvascular responses in coronary and gracilis arterioles isolated from control and obese animals. First we detected angiotensin 2 (Ang 2) and norepinephrine induced vasoconstrictor responses. Half an hour after the first application we rerecorded the agonist induced responses. The Ang 2 and norepinephrine induced repeated responses were also registered in the presence of Rho kinase inhibitor Y27632. We used acetylcholine (ACh) for the assessment of endothelial function. The ACh evoked

responses were also registered after half an hour incubation with the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME). To assess EDHF mediated response we registered coronary arteriolar dilations in the simultaneous presence of L-NAME and the cyclooxigenase inhibitor indomethacin. The ACh evoked responses were also observed in the presence of BKCa inhibitor iberiotoxin, small conductance calcium activated potassium channel (SKCa) inhibitor apamin and the intermediate conductance calcium activated potassium channel (IKCa) inhibitor TRAM-34. Dilator responses for the specific BKCa opener NS-1619 were also observed in isolated coronary arterioles. For the disruption of caveolae we incubated vessels *in vitro* with methyl-β-cyclodextrine for 90 minutes.

3.5 Detection of reactive oxygen species in the vascular wall

The generation of reactive oxygen species by the vessel wall was determined by Amplex Red Assay Kit. In the reaction the extracellularly generated H_2O_2 was detected with horse radish peroxidase linked Amplex Red fluorescent assay.

3.6 Measurement of Rho kinase activity

First order branches of femoral arteries were homogenized in ice cold buffer, then the protein content was detected. The arterial lysates with similar protein contents were placed in factory designed substrate containing wells. After incubation with the horse radish peroxidase labelled phospho specific antibody, the substrate was added, and the reaction evoked changes in colour was detected by a spectrophotometer.

3.7 Immunohistochemistry

After embedding the hearts obtained from control and HFD rats, 10 µm thick consecutive sections were created. After fixing with acetone, the slides were incubated with polyclonal anti-cav-1 and anti-smooth muscle actin or with polyclonal anti-BKCa and anti-smooth muscle actin antibodies. The nuclei were visualized by DAPI.

3.8 Western immunoblot

We detected BKCa and cav-1 expression in septal coronary arteries isolated from control and HFD rats.

3.9 Statistics

We used Student type t test in our statistical analyses. The level of significance was determined to be a $p<0.05$.

4. RESULTS

4.1 Skeletal muscle arteriolar function in obesity

Constriction of gracilis arterioles in obese Zucker rats

The weight of the obese Zucker rats was significantly higher then the LZ rats. In gracilis arterioles isolated from control animals Ang 2 elicited dose dependent constrictions, which was significantly diminished for the second application after half an hour incubation. There was no difference in the magnitude of norepinephrine induced constriction between the first and the second application. The OZ arterioles showed a significantly larger constriction then the LZ arterioles. The gracilis arterioles isolated from OZ rats showed a significantly increased constriction for the second application of Ang 2. The homogenizates of OZ femoral arteries showed significantly increased Rho kinase activity when compared to those of controls. In isolated LZ arterioles the Rho kinase inhibitor Y27632 did not have any effect neither on the first nor on the second response to angiotensin 2. In comparison, the isolated OZ gracilis arterioles in the presence of Y27632 demonstrtaed decreased constriction to the first application of Ang 2, whereas the second constriction response was almost completely abolished. Incubation of the vessels with Y27632 did not alter the responses to norepinephrine in any of the two groups.

Endothelium dependent dilation of gracilis arterioles in high fat diet fed mice

High fat diet induced significant weight gain in both the wild type ($Nrf2^{+/+}$) and the Nrf2 ($Nrf2^{-/-}$) knockout mice. The weight gain was lower in the mice that were fed with the resveratrol modified high fat formula. High fat diet induced impairment of the ACh evoked

endothelium dependent dilation in both $Nrf2^{+/+}$ and $Nrf2^{-/-}$ mice. Supplementation of resveratrol in the high fat diet restored ACh dilation to the level observed in control diet $Nrf2^{+/+}$ mice. Interestingly, addition of resveratrol to the high fat diet only resulted in partial restoration of the ACh dilation in the $Nrf2^{-/-}$, so the observed difference in the magnitude of ACh dilation when compared to $Nrf2^{-/-}$ mice on control diet still remained. High fat diet significantly enhanced the production of reactive oxygen species (ROS) by the vascular wall in $Nrf2^{+/+}$ as well as in $Nrf2^{-/-}$ mice. In $Nrf2^{+/+}$ mice resveratrol supplementation restored vascular ROS production to the level observed in mice on control diet, however it only reduced the ROS production partially in the $Nrf2^{-/-}$ group.

4.2 Coronary arteriolar function in obesity

After commencing the high fat diet for 10 weeks the body weight, the serum insulin, glucose and total cholesterol of the rats was significantly higher then those on standard diet. Surprisingly we were not able to detect any difference in the magnitude of ACh dilation between the control and HFD coronary arterioles. The NO sythase inhibitor L-NAME reduced the ACh induced dilation in the control coronary arterioles, but it had no effect on arterioles isolated from HFD rats. There was no difference in the ACh evoked dilation in the 2 groups after simultaneous incubation of the vessels with L-NAME and the cyclooxigenase inhibitor indomethacin. Interestingly the BKCa opener NS-1619 induced a significantly greater dilator response in the coronary arterioles isolated from HFD rats when compared to controls. The BKCa inhibitor iberiotoxin markedly reduced arteriolar dilations in the HFD group, as well as it had no effect on control coronary arteriolar responses. The SKCa inhibitor apamin and the IKCa inhibitor TRAM-34 significantly reduced the ACh induced dilations in both groups. The

immunohistochemical sections detected the BKCa protein expression in the smooth muscle cell layer, which was characterized by co-staining with α-actin. We observed marked reduction in cav-1 staining in the HFD sections. We also detected a decreased cav-1 protein expression in HFD septal coronary arteries, but we did not detect any significant change in the the expression of BKCa channels.

4.3 Revealing the underlying mechanisms responsible for the preserved coronary dilation

To provide functional evidence for the interaction between cav-1 and BKCa channels control coronary arterioles were incubated with Methyl-β-Cyclodextrine (MβCD), which is known to disrupt caveolae structure. Interestingly in the presence of MβCD iberiotoxin inhibited the ACh induced dilation and NS-1619 evoked responses were also augmented, similar to the findings observed in high fat diet fed rats. To gain further proof regarding the interaction between the cav-1 and the BKCa channels we isolated arterioles from cav-1 knockout mice. In arterioles isolated from cav-1 knockout mice iberiotoxin abolished ACh induced dilation as well as we found an augmented NS-1619 induced dilation, when compared to ones found in wild type mice.

5. DISCUSSION

Obesity is a pathological state associated with high cardiovascular morbidity and mortality, being an emerging health problem throughout the world. It is well known that in obesity atherosclerosis develops in the large conduit vessels. Recent studies have made it clear that one can observe alterations in the microcirculation well before the development of dysfunction in the large vessels, and this microvascular dysfunction is also an important cardiovascular risk factor associated with obesity. Despite the large number of clinical and experimental studies, the nature and the role of obesity associated microvascular changes still remains unclear. Therefore in my experiments I aimed to study the microcirculation of different tissues. In order to investigate this claim we isolated skeletal muscle and coronary arterioles from different types of animal models of obesity and assessed endothelial and smooth muscle function.

5.1 Constriction of gracilis arterioles in obese Zucker rats

In our first set of experiments we detected vasoconstrictor responses in response to repeated administration of Ang 2 in gracilis arterioles isolated from obese Zucker rats. Obesity is often associated with hypertension, that can develop in response to the increased activation of the renin-angiotensin system. Our group previously reported elevation in systolic blood pressure in high fat diet induced obesity. Previously published data shows that angiotensin converting enzyme inhibitor treatment improved obesity associated endothelial dysfunction in OZ rats. Publications from several different labs provide evidence that an increased Ang 2 response can lead to increased peripheral resistance and the subsequent

development of hypertension. In our experiments we observed a significantly increased Ang 2 response in arterioles obtained from OZ rats, when compared to controls. We raised the hypothesis that the increased Ang 2 induced constriction is due to sustained functional availability of the type one angiotensin 2 (AT1) receptors. It is known, that the AT1 receptors are continously migrating between the cell surface and the endoplasmic reticulum. This process determines the number of the active AT1 receptors available for the next stimuli, and regulates the availability of AT1 receptors. In our experiments we hypothesized that the physiological balance in the movement of the AT1 receptors is altered in obesity, that can lead to sustained AT1 receptor availability and consequently to increased vasoconstriction. To detect the function of the readily available, active AT1 receptor we reassessed Ang 2 evoked responses following half an hour equlibrium after the first response. Under physiological conditions repeated application of angiotensin leads to tachyphylaxis. The underlying mechanisms is Ang 2 induced desensitisation and consequent internalisation of the AT1 receptors. Indeed in LZ arterioles we detected a significantly reduced constriction in response to the second application of Ang 2. Interestingly in gracilis arterioles of OZ rats we observed a sustained Ang 2 constriction in response to the second stimuli, which indicated the lack of tachyphylaxis. Because we found no tachyphylaxis regarding the phenylephrine evoked responses in the OZ arterioles, we conclude that observed difference is not due to alterations in the smooth muscle contractile system, such as changes in the calcium sensitivity. Because in our experiments the OZ arteries showed elevated Rho kinase activity when compared to the LZ ones, we raised the hypothesis that the Rho kinase signalling pathway can participatein the internalisation of AT1 receptors. To elucidate this claim we observed the Ang 2 evoked responses in the presence of Rho kinase

inhibition. Interestingly Rho kinase inhibitor incubation reduced the previously sustained Ang 2 repeated responses in isolated OZ gracilis arterioles.

Summerizing we can tell, that in the genetic model of obesity the increased activation of the Rho kinase can lead to sustained vasoconstriction to the repeated administration of angiotensin 2.

5.2 Endothelium dependent dilation of gracilis arterioles in high fat diet fed mice

In the next set of experiments we induced obesity in mice by feeding them with high fat diet. In response to the high fat diet the isolated gracilis arterioles exhibited diminished ACh mediated dilation when compared to the ones iolasted from sedentary animals. Interestingly when the high fat diet was supplemented with resveratrol, the abovementioned impairment in the ACh response diminished, that is similar to findings observed by other groups. We investigated the effect of high fat diet and resveratrol in NRf2 knockout animals as well. The Nrf2 transcription factor is responsible for the regulation ofseveral gene transciption, among one can find several antioxidant enzymes. The isolated gracilis arterioles of control diet Nrf2 knockout mice showed no impairment of endothel dependent dilation, when compared to dilations of wild type microvessels. Based on this we concluded, that under physiological conditions NRf2 transcription factor does not play a major role in the regulation of ACh evoked responses. In the Nrf2 knockout mice the high fat diet caused a greater reduction in the magnitude of ACh dilation then it caused in the wild type group, so we draw the conclusion, that Nrf2 is protective against the harmful effect of high fat diet. Also resveratrol treatment induced only partial restoration of the ACh induced dilations in $Nrf2^{-/-}$ mice fed with high fat diet. This suggests, that the Nrf2 may play

an important role in the vasoprotective effect of resveratrol. The ROS production is a major component of the high fat diet induced deleterious changes in the microvascular wall. Our group has previously shown in high fat diet induced obesity that xanthine oxidase derived superoxide impairs the NO mediated dilation of isolated gracilis arterioles. Based on this we beleived that femoral aretrial homogenizates of mice kept on high fat diet will increase ROS production, that is ameliorated by treatment with resveratrol. Our results show that high fat diet induces elevation in the ROS level both in the $Nrf2^{+/+}$ and in the $Nrf2^{-/-}$ groups. Resveratrol supplementation in the $Nrf2^{+/+}$ high fat diet mice reduced the vascular ROS production to similar amount that was observed in control levels. Based on this it is likely, that resveratrol exerts its vasoprotective effect via inhibition of ROS generation. In contrast with these findings resveratrol reduced vascular ROS production only partially in the high fat diet $Nrf2^{-/-}$ group.

To sum up our findings, we found that high fat containing diet induced impairment in the endothelium mediated dilation in isolated skeletal muscle arterioles. Resveratrol can ameliorate high fat diet induced microvascular dysfunction via the activation of Nrf2 transcription factor and subsequent reduction in the production of vascular reactive oxygen species.

5.3 Coronary arteriolar function in obesity

In our next set of experiments we induced obesity by feeding rats wit high fat diet (HFD). Previously our group described that high fat diet induced impairement in the vasodilation of isolated skeletal muscle arterioles. Interestingly in our present study in coronary microvessels isolated from the HFD group we found a preserved ACh induced vasodilation when compared to dilation of control arterioles. Similar to

our findings certain publications reported not only preserved but increased coronary arteriolar dilations in different types of animal models of obesity. In our experiments we also detected that blockade of the NO synthase decreased the magnitude of dilation in control vessels, but did not have any effect on HFD arterioles. This suggests, that obesity can impair NO mediated dilatory responses in the coronary microcirculation, but the impairment in vasodilatory function can be compensated via undetermined compensatory mechanisms. In our experiments we also provided evidence, that the EDHF mediated dilation is also preserved in coronary arterioles of obese rats. Our data also show that in HFD coronary arterioles BKCa channels actively participate in the EDHF response of coronary arterioles, and that the BKCa channel opener caused a significantly greater response in coronary arterioles isolated from HFD animals. This suggests that BKCa channels can be responsible for the compensated coronary arteriolar vasodilator response in obesity. It is known for a long time, that BKCa channels are parts of a macromolecular signalling complex, that includes several enzymes and ion channels. Cell culture experiments provided evidence, that BKCa channels are located into caveolae, and caveolin-1 exerts a negative regulatory effect on the activation of BKCa channels by isoproterenol. Based on these findings we raised the hypothesis that a decreased inhibitor effect of the caveolin-1 can be responsible for the facilitated BKCa mediated dilation in the high fat diet fed animals. Interestingly we found that cav-1 immunostaining was pronouncedly reduced in coronary arterioles of HFD rats. In connection to this we also detected a reduced cav-1 protein expression in isolated coronary arteries of HFD animals, when compared to HFD group. To get further evidence about the inhibitory effect of cav-1 on BKCa channels, we performed additional functional experimentson isolated arterioles obtained from cav-1 knockout mice. Interestingly

BKCa channels showed to have a marked contribution to the EDHF responses in arterioles isolated from cav-1 KO animals , where it was absent in wild type mice. Theoretically speaking by disrupting the caveolae one would be able to mimic the effect of the lack of caveolae on BKCa channel function. Accordingly we detected an increase in the BKCa channel participation in the EDHF arteriolar responses when arterioles were exposed to *in vitro* MβCD treatment.

To sum up we were the first to provide functional evidence fro the negative regulator function of cav-1 exerted on BKCa channels in intact vessels, that can provide reasonable explanation for the observation that BKCa channels do not participate in the EDHF response under physiological conditions. In high fat diet induced obesity the loss of caveolin-1 can result in the gain of function of the BKCa channels. This process seems to be essential in order to preserve coronary arteriolar dilator function in obesity.

6. SUMMARY

It is well known that resistance vessel vasomotor function is altered in obese conditions, however the underlying mechanisms are not completely understood. Therefore, we aimed to study the vasomotor function in isolated skeletal muscle and coronary arterioles in animal models of obesity. Our key observations were the following: 1) Obesity is associated with sustained constriction in response to repeated application of angiotensin 2 in isolated skeletal muscle arterioles, likely due to an activation of the Rho kinase pathway. 2) Impairment of endothelium mediated vasodilation in skeletal muscle arterioles isolated from obese rodents can be prevented by resveratrol administration, likely via Nrf2 transcription factor activation and consequent inhibition of reactive oxygen species generation. 3) Coronary arterioles exhibit preserved endothelium mediated dilation in obesity. BKCa channels gain function in mediating EDHF dependent vasodilator responses via the loss of inhibition by caveolin-1, a process that seems to be essential for preserving coronary microvascular function. Based on our findings, we believe that vascular responses can be impaired in the skeletal muscle microcirculation, while coronary arterioles can actively adapt to the higher metabolic demand. We believe that different vasodilator agents acting on BKCa channels, Rho kinase, or resveratrol play a potential role in the future treatment of obesity associated microvascular dysfunction.

Register Number: DEENKÉTK /186/2011.
Item Number:
Subject: Ph.D. List of Publications

Candidate: Attila Fehér

Neptun ID: IUX2O4

Doctoral School: Kálmán Laki Doctoral School

List of publications related to the dissertation

1. Bagi, Z., **Fehér, A.**, Cassuto, J., Akula, K., Labinskyy, N., Kaley, G., Koller, Á.: Increased availability of angiotensin AT1 receptors leads to sustained arterial constriction to angiotensin II in diabetes - role for Rho-kinase activation.
   Br. J. Pharmacol. 163 (5), 1059-1068, 2011.
   DOI: http://dx.doi.org/10.1111/j.1476-5381.2011.01307.x
   IF:4.925 (2010)

2. **Fehér, A.**, Rutkai, I., Beleznai, T., Ungvári, Z., Csiszár, A., Édes, I., Bagi, Z.: Caveolin-1 limits the contribution of BK(Ca) channel to EDHF-mediated arteriolar dilation: implications in diet-induced obesity.
   Cardiovasc. Res. 87 (4), 732-739, 2010.
   DOI: http://dx.doi.org/10.1093/cvr/cvq088
   IF:6.051

3. Ungvári, Z., Bagi, Z., **Fehér, A.**, Recchia, F.A., Sonntag, W.E., Pearson, K., de Cabo, R., Csiszár, A.: Resveratrol confers endothelial protection via activation of the antioxidant transcription factor Nrf2.
   Am. J. Physiol.-Heart Circul. Physiol. 299 (1), H18-H24, 2010.
   DOI: http://dx.doi.org/10.1152/ajpheart.00260.2010
   IF:3.88

4. Jebelovszki, É., Király, C., Erdei, N., **Fehér, A.**, Pasztor, T. E., Rutkai, I., Forster, T., Édes, I., Koller, Á., Bagi, Z.: High-fat diet-induced obesity leads to increased NO sensitivity of rat coronary arterioles: Role of soluble guanylate cyclase activation.
   Am. J. Physiol. Heart. Circ. Physiol. 294 (6), H2558-H2564, 2008.
   DOI: http://dx.doi.org/10.1152/ajpheart.01198.2007
   IF:3.643

List of other publications

5. Bagi, Z., **Fehér, A.**, Cassuto, J.: Microvascular responsiveness in obesity: Implications for therapeutic intervention.
   Br. J. Pharmacol. Epub ahead of print (2011)
   DOI: http://dx.doi.org/10.1111/j.1476-5381.2011.01606.x
   IF:4.925 (2010)

6. Beleznai, T., **Fehér, A.**, Spielvogel, D., Lansman, S.L., Bagi, Z.: Arginase 1 contributes to diminished coronary arteriolar dilation in patients with diabetes.
   Am. J. Physiol.-Heart Circul. Physiol. 300 (3), H777-H783, 2011.
   DOI: http://dx.doi.org/10.1152/ajpheart.00831.2010
   IF:3.88 (2010)

7. Bagi, Z., **Fehér, A.**, Beleznai, T.: Preserved coronary arteriolar dilatation in patients with type 2 diabetes mellitus: Implications for reactive oxygen species.
   Pharmacol. Rep. 61 (1), 99-104, 2009.
   IF:2.086

8. Rutkai, I., **Fehér, A.**, Erdei, N., Henrion, D., Papp, Z., Édes, I., Koller, Á., Kaley, G., Bagi, Z.: Activation of prostaglandin E2 EP1 receptor increases arteriolar tone and blood pressure in mice with type 2 diabetes.
   Cardiovasc. Res. 83 (1), 148-154, 2009.
   DOI: http://dx.doi.org/10.1093/cvr/cvp098
   IF:5.801

Total IF: 35,191

Total IF (publications related to the dissertation): 18,499

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

06 September, 2011