SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Vascular effect of some anesthetic and intensive therapeutic agents

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The Examination takes place at Institute of Immunology, Life Science Bldg, room 2.209-211 Faculty of Medicine, University of Debrecen, 6th of December, 2018, 11:00 a.m.

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The PhD Defense takes place at the Life Science Bldg. Lecture Hall F008-009, Faculty of Medicine, University of Debrecen, 6th of December, 2018, 13:00

1 Introduction

1.1 The role of the endothelium and smooth muscle cells in the regulation of microcirculation

Endothelial cells lining the inner surface of blood vessels were considered as a simple mechanical barrier until 1970s. Since then a more complex role of these cells is emerging. Several important physiological functions can be attributed to endothelial cells such as regulation of the vascular tone, they play a primary role in the regulation of vascular permeability, thrombocyte aggregation, inflammatory and immunological processes, metabolic processes and endocrine regulation.

The cumulative surface area of the endothelium lining arterial, venous and lymphatic vessels is ~1000 m^2, therefore, these cells play a unique role in the in the exchange of information and material between lumen of the vascular system and tissues/cells surrounding them. The structure and function of the endothelium shows great variability among species, gender, type of the vessel, its localization within the body and the order of the vessel in the vascular branch. A given mechanism/process is only characteristic for a blood vessel originating form a given organ of a given species, and of a given order in the vasculature. This latter fact makes experimental observation of the function of blood vessels very difficult and challenging to compare the various results. Apart from this endothelium was in the center of physiological research in the last two decades whereby complex regulatory processes were evaluated and deciphered step-by-step. However, there are still many unanswered questions.

Endothelial cells and the smooth muscle cells lying underneath form a functional unit. The result of their interaction is the vascular tone, maintained by smaller arteries and arterioles (=resistance vessels), which determines the local, organ level and generalized blood flow.

The mutual interaction of endothelial cells and vascular smooth cells (VSMCs) is supported clearly by the fact that the diameter of blood vessels having intact endothelium is significantly larger than that of the same vessels with endothelium stripped off. The endothelium can influence the function of the underlying smooth muscle cells through a variety of mechanisms which includes a direct link between endothelial cells and muscle cells through gap junctions, or synthesis and release of mediators inducing constriction or relaxation of the smooth muscle cells, such as nitrogen-monoxide (NO), breakdown products of arachidonic acid, proteins/peptides (e.g. endothelin), purine derivatives, adenosine, reactive oxygen species, and vasoactive hormones e.g. angiotensin II. The ability of the

endothelium to regulate the local microcirculation requires a "sensory" function of micro vessels whereby the alterations of the local blood flow are sensed by membrane potential-dependent and independent mechanisms. This will allow the endothelium to regulate the blood flow of a tissue or organ according to the actual needs, independent of the generalized vascular tone. This delicate regulatory mechanism is impaired in endothelial disfunction where the alteration of the synthesis and release vasoconstrictor and vasodilator compounds may lead to the development of cardiovascular diseases (e.g. hypertonia, atherosclerosis, cardiovascular senescence).

There are three major mechanisms for endothelium-derived vasodilation: the NO- dependent, the prostacyclin-dependent and the Endothelium Derived Hyperpolarizing Factor (EDHF)-dependent vasodilation.

Of these mechanisms I am focusing on the vasoregulation responses mediated by NO and EDHF in the dissertation, the prostacyclin pathway was pharmacologically inhibited in all experiments. The subsequent chapters aim at summarizing the abundant and very complex literature regarding the two mechanisms studied here.

1.1.1 Production of NO and its role in vasodilation

The NO-dependent vasodilation response starts with the activation of the endothelial NO synthase (eNOS). The enzyme generates nitrogen-monoxide and L-citrulline from L-arginine and molecular oxygen. NO then diffuses into the vascular smooth muscle cells underlining the endothelium and activates the soluble guanylate cyclase to produce cGMP. cGMP then will activate in vascular smooth muscle cells the cGMP-dependent protein kinase G I (PKGI) which leads to the decrease in the cytosolic free Ca^{2+} concentration by two mechanisms. On one hand PGKI will phosphorylate the large conductance Ca^{2+} activated K^+ channels (BK_{Ca} = $K_{Ca}1.1$ according to the universal nomenclature) which increases the open probability of the channel thereby leading to hyperpolarization of the membrane. This latter inhibits the activity of voltage-gated Ca^{2+} channels and cause a reduced influx of extracellular Ca^{2+} into the VSMC. In addition, PKGI-dependent increase in activity of the sarco-endoplasmic reticulum Ca^{2+}-ATP-ase (SERCA) leads to an increased rate of Ca^{2+} transport into the endoplasmic reticulum which also leads to the decrease in the cytosolic free Ca^{2+} concentration of VSMCs. These mechanisms combined with the PKGI-dependent decrease in the Ca^{2+} sensitivity contractile proteins lead to the relaxation of the VSMCs. In addition, NO also relaxes VSMCs in a PKGI-independent pathway: NO acts directly on SERCA and on the

plasma membrane K^+ channels (BK_{Ca}, Kv, K_{ATP}, K_{IR}) thereby contributing to the relaxation of the blood vessels.

1.1.2 The role of EDHF in the regulation of vascular tone

1.1.2.1 The development of the EDHF concept in the last decades

Beyond NO and prostacyclin, the hypothesis for the existence of a third endothelium-derived vasodilator factor has been emitted because it has been observed that, despite the effective inhibition of NO and prostacyclin pathways, a remaining endothelium-dependent relaxation persists in most of the vascular beds. Because vascular smooth muscle cells hyperpolarization is an absolute prerequisite for this relaxation, this factor has been termed endothelium-derived hyperpolarizing factor (EDHF).

Since these observations, the endothelium-dependent hyperpolarization phenomenon, has been extensively studied in animal models, it is now generally admitted that, depending on the species, vascular beds and endothelial stimuli used, several endothelium-derived factors including NO and prostacyclin themselves, K^+, H_2O_2 and other reactive oxygen species can hyperpolarize the underlying smooth muscle through different mechanisms.

1.1.2.2 Pathways for EDHF, the role of different K^+ channels in the EDHF mechanism

Although the EDHF mechanism is still not fully resolved it is commonly believed that EDHF can hyperpolarize the VSMCs in three principal pathways.

1./ EDHF can passively diffuse from the endothelium to activate calcium-activated potassium (K_{Ca}) channels of large conductance (BK_{Ca}) located on the smooth muscle cells thereby promoting the release of K^+ and membrane hyperpolarization.

2./ EDHF can act in an autocrine manner to facilitate the activation of the endothelial K_{Ca} channels of small (SK_{Ca}) and intermediate (IK_{Ca}) conductance directly mediated by Ca^{2+} inducing the release of K^+ and the hyperpolarization of the endothelial cells. K^+ released from the endothelial cells into the myoendothelial space through SK_{Ca} and IK_{Ca} channels activates the Na^+/K^+ ATPase and the inward rectifying potassium channels (K_{IR}) located on the smooth muscle cells promoting the release of K^+ and subsequent hyperpolarization of VSMCs.

3./ The third pathway is based on the hyperpolarization of the endothelial cells mentioned in point 2./ - this hyperpolarization is transmitted electronically through the myoendothelial gap junctions into the smooth muscle cell layer leading to the hyperpolarization of the VSMCs.

Hyperpolarization of VSMCs decreases the open-probability of voltage-gate Ca^{2+} channels thereby lowering cytosolic free Ca^{2+} concentration and thus, provoking vasorelaxation.

Although the EDHF-dependent vascular relaxation mechanisms are present in all vascular beds of all species, it has been concluded that the relative contribution of the EDHF mechanism to vasoregulation increases with the decrease of the vessel diameter.

1.2 The effect of anesthetic agents on the microcirculation

Most of the *in vivo* experiments in microcirculation research are conducted in anesthesia. This raises a simple and logical question: does the anesthetic agent applied influence microcirculation and the function of the resistance arterioles, i.e., if the outcome of the experiment is influenced by the anesthetic agent chosen.

The currently used volatile anesthetic agents (halothane, isoflurane) decrease the vascular resistance, although the exact mechanism is missing. Experiments did not support the previous hypothesis that volatile anesthetics increase NO production of endothelial cells. At the same time inhibition of the agonist-induced endothelium-dependent relaxation was described in aortic rings in the presence of volatile anesthetic agents. According to the current theory volatile anesthetics inhibit endothelium-dependent vasodilatation, but at the same time, they facilitate endothelium-independent relaxation of VSMCs. This latter effect can be attributed to the decreased availability of intracellular free Ca^{2+} and/or the decreased Ca^{2+} sensitivity of the contractile apparatus in the presence of volatile anesthetics.

As a consequence, these two complementary mechanisms may lead to the loss of the vascular tone. The experimental findings mentioned above can also be attributed to the direct effect of volatile anesthetics on the K^+ channels and/or the gap junctions of endothelial cells.

Other anesthetic drugs applied in the current study (ketamine, pentobarbital) may also influence the vascular tone, however, the role of the endothelium is still unclear in this process. Ketamine increases vascular tone which might be attributed to the increase in the catecholamine release. On the other hand, the inhibition of endothelium-dependent vasodilatation was also shown in the presence of ketamine *in vitro* through the inhibition of NO and EDHF mechanisms. This effect of ketamine is characteristic for its R(-) stereoisomer.

1.3 Vasoactive properties of levosimendan and its metabolite, OR-1896, *in vivo*

The Ca^{2+}-sensitizer positive inotropic agent levosimendan was developed for the treatment of decompensated heart failure. The drug also causes a strong vasodilatation of the peripheral blood arteries and veins (inodilator). Since these effects of the drug are independent of beta adrenergic receptors levosimendan can also be used in parallel to beta blocker therapy. The initial optimism, fueled by the promising improvement in short-term outcome of early clinical trials in patients with decompensated heart failure (LIDO) or developing heart failure acute myocardial infarction (RUSSLAN) has been tempered by less favorable impact on long-term outcome in the large-scale clinical trials SURVIVE and REVIVE. Nevertheless, the results of recent meta-analyses offer encouraging perspectives on the usefulness of levosimendan in circumstances of acute heart failure.

In order to support the clinical applicability of levosimendan delineating the molecular mechanism of its action is absolutely required both *in vitro* and *in vivo*. The mechanism of action of levosimendan is complex as it involves an active long-lived metabolite, OR-1896 (the (−) enantiomer of N-[4-(1,4,5,6-tetrahydro-4-methyl-6-oxo-3-pyridazinyl) phenyl] acetamide), and also involves interactions with more than one molecular target within the cardiovascular system.

This complex effect of the levosimendan may explain that in addition to the strong positive inotropic effect the drug acts as a strong vasodilator and may be cardioprotective as well.

The molecular background of the Ca^{2+}-sensitizing effect of levosimendan relates to its specific interaction with the Ca^{2+}-sensor troponin C molecule in the cardiac myofilaments. Its positive inotropic effect in the heart develops in combination with the vasodilation of peripheral and coronary arteries and of veins. The vasodilation induced by levosimendan or OR-1896 have been found to be mediated by ATP-sensitive potassium (K_{ATP}) channels and possibly other potassium channels (e.g., large-conductance Ca^{2+} - activated potassium (BK_{Ca}) channels and/or voltage-sensitive potassium channels).

Previous studies indicated that the infusion of rats with levosimendan or OR-1896 resulted in significant reductions in systemic peripheral vascular resistance, suggesting that these drugs exert their vasodilator effects predominantly on resistance-sized vessels. However, the vasomotor effects of levosimendan and OR-1896 on real resistance arteries *in vivo* have not yet been investigated.

Our present aim was therefore, to characterize the microvascular effects of levosimendan and OR-1896 *in vivo*, and hence, to provide evidence as to their vasodilator roles in the microcirculation. The changes in the diameters of third order-cremaster muscle arterioles (diameter: ~ 20 μm) in the presence of increasing concentrations of levosimendan or OR-1896 were monitored by means of intravital videomicroscopy. This allowed a comparative assessment of the vasodilator effects induced by levosimendan and OR-1896 in the microcirculation *in vivo*. Since levosimendan and OR-1896-induced vasodilation has been shown to be associated with various degrees of K_{ATP} channel, K_{ATP} channel function modulators were also employed.

2 Aims

1. To identify the role of K_{IR} channels in resistance arterioles of cremaster muscle of rats in vivo. The cremaster muscle of rats is a widely used model system for studying microcirculation *in vivo*. The mechanism responsible for the vasodilatation of resistance arterioles induced by modest increase in the extracellular K^+ concentration was unclear at the time of the experiments. We hypothesized that inward rectifying potassium channels (K_{IR}) can play a role in this process, however, the existence and functional role of these channels in resistance arterioles has not been clarified *in vivo*. Consequently, we tested if pharmacological inhibition of the K_{IR} channels may influence the vasodilatation response of resistance arterioles in rat cremaster muscle induced by increase in the extracellular K^+ concentration, *in vivo*.

2. To test the hypothesis that various anesthetic agents differentially influence microcirculation in the rat cremaster muscle in vivo. In vivo experiments require anesthesia of the experimental animals. This raised the question if the choice of the anesthetic agent (isoflurane, halothane ketamine) may influence microcirculation in the model system. Our hypothesis was that various anesthetic agents may alter the agonist-induced (acetylcholine, bradykinin) endothelium-dependent vasodilation response of resistance arterioles mediated by NO and EDHF pathways. We tested this hypothesis experimentally in rat cremaster muscle resistance arterioles *in vivo* using pharmacological inhibition of the NO and EDHF pathways.

3. To characterize the effect of levosimendan and OR-1896 on the microcirculation in cremaster muscle in vivo and determine if these compounds act as vasodilators. Previous *ex-vivo* experiments showed that levosimendan and its long-lived metabolite OR-1896 are potent vasodilators in blood vessels having larger diameter than resistance arterioles. This response was attributed to K_{ATP} channels. We hypothesized that K_{ATP} channels may also play a role in the vasoactive effects of levosimendan and OR-1896 in the regulation of microcirculation in resistance arterioles *in vivo*. We tested this hypothesis in rat cremaster muscle resistance arterioles *in vivo* using intravital microscopy.

3 Materials and methods

3.1 Experimental animals

All experimental procedures were approved by the institutional Animal Care and Use Committee, in compliance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes. Experiments were carried out on female Wistar rats (200g-300g). Animals were anesthetized using ketamine, halothane, isoflurane or sodium pentobarbital. At the end of the experiments the animals were euthanized by an injection of sodium pentobarbital (150 mg/kg).

3.2 Rat Anesthesia, Vascular Access, and Monitoring

Halothane and isoflurane anesthesia: The animal was placed in a closed chamber through which the volatile anesthetic was passed at flow rates sufficient to prevent CO_2 accumulation. After anesthesia induction, the trachea was cannulated. Animals were paralyzed with pancuronium bromide (1 mg/kg iv) and mechanically ventilated (total inspired O_2 = 30%) to maintain PO, and PCO, at normal values (~100 and 35 mmHg, respectively), as determined by arterial blood gas analysis. Catheters were placed in the carotid artery and jugular vein for direct blood pressure monitoring and drug administration, respectively. Body temperature was maintained at 37°C using a heating. Halothane (1.2%) or isoflurane (1.4%) were administered through appropriate temperature- and flow-compensated vaporizers. Ketamine anesthesia was induced by intraperitoneal injection (150 mg/kg) and maintained by continuous intravenous administration of the drug at the rate of 1.5 mg/kg/min. These techniques produce approximately equivalent and moderate levels of anesthesia in rats.

Sodium pentobarbital anesthesia was initiated by a subcutaneous injection of sodium pentobarbital (50 mg/kg). A constant level of anesthesia was maintained throughout the experiments by the subcutaneous injection of supplemental doses (20% of the original dose) of the anesthetic agent every 30–45 min. The trachea was cannulated to facilitate respiration and the anesthetized rat was placed on a heated platform

3.3 Cremaster Muscle Preparation

The left cremaster muscle was exposed through a midline scrotal incision as described in the literature. The anesthetized rat was placed on a platform, and the cremaster muscle, with nerves and vessels intact, was spread over a heated, transparent pedestal. The whole preparation was then placed on the x–y stage of a microscope and superfused continuously with warmed Krebs buffer at 2 ml/min. The Krebs buffer consisted of 132 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 1.2 mM $MgSO_4$ and 2 mM $CaCl_2$. The buffer was bubbled with 95% N_2-5% CO_2. After the surgical procedure the preparation was allowed to equilibrate for at least 30 min before the start of the experimental protocol.

The vascular supply to the cremaster muscle enters through a central artery that branches successively. Each branch from the central artery was defined as a specific central artery that branches successively. Each branch from the central artery was defined as a specific "order," with the central vessel designated first order (A1), the first branch as second order (A2), and so on. We studied fourth-order arterioles (average diameter: 20-30μm.)

3.4 Intravital microscopy of the rat cremaster muscle

Cremaster muscle arterioles were observed using a high-resolution television camera (Microimage Video CA2063) mounted on the microscope. The microscope image was displayed on a video monitor (Sony PVM-1343MD) and videotaped (Panasonic AG1970). For analysis, the image was displayed on the monitor, and vessel diameters were measured every 30 s using an electronic video image analyzer (Boeckeler VIA-150) during and after each drug treatment.

During the third set of experiments images were collected with a CCD camera and were recorded on a VHS recorder. The internal arteriolar diameters were measured offline with the aid of videocalipers (Colorado Instruments, USA), by two blinded, independent investigators.

3.5 Characterization of agonist-induced vascular responses

The muscle was superfused with the Krebs solution for 30-60 min. flowing the surgical procedure in order to equilibrate the system and obtain stable vessel diameters. The vital parameters of the experimental animal were continuously monitored (intraarterial blood pressure measurement and blood gas analysis) and kept within the physiological range.

Only one vessel was studied in each animal. All vessels chosen for study were then exposed to 100 μM adenosine (ADO). Vessels that did not respond to ADO challenge with a robust vasodilation (at least 50% increase above base- line) were not studied further. ADO was diluted in the superfusion solution and infused slowly via an injection port into the superfusion line to achieve the desired concentration for 1 min. Vessel diameters were measured every 30 s. At least 10 min elapsed between each drug superfusion. This time was sufficient for blood vessel diameters to react to the treatments and to return to baseline after washout. When multiple drug treatments were applied the order of the drug administration was randomized

3.6 Experimental protocols

3.6.1 Elucidating the role of K_{IR} channels in the extracellular K^+-induced vasodilatation of rat cremaster muscle resistance arterioles.

The function of the resistance arterioles was tested using adenosine (ADO) thereafter the superfusion of the Krebs buffer was switched to a buffer having high [K^+]. High [K^+] buffers were prepared by substituting KCl for NaCl in the normal buffer recipe such that the desired [K^+] of 10 and 60 mM was obtained. To prevent any possible effect of sequential addition, the order of administration of different [K^+] solutions was randomized for each experiment. The muscle was exposed to both [K^+] for 10 min and diameters were recorded. In preliminary experiments we found that arteriolar diameter stabilized at a new value within 5 min after exposure to a new [K^+]. Therefore, the reported diameter is the average diameter over the final 5 min of exposure to a given [K^+]. K^+-channel inhibitors (1 μM glibenclamide; 100 nM, iberiotoxin (IBTX); 50 μM, $BaCl_2$) were added to the superfusate for 30 min prior to re-exposure to increased [K^+]. At the concentrations used, these drugs are relatively specific blockers of the activities of K_{ATP}, $K_{Ca}1.1$, and K_{IR} channels, respectively. Only one inhibitor per animal was used. Each rat was used as its own control to compare the effect of different [K^+] in the absence and then the presence of a K^+ channel blocker.

3.6.2 Elucidating the role of anesthetic agents on the NO and EDHF pathways during agonist-induced vasodilatation in cremaster muscle resistance arterioles

All animals were treated with indomethacine (5mg/kg, intravenous) following the cremaster muscle preparation. This dose of indomethacine inhibits prostaglandin synthesis in rats efficiently according to the relevant literature.

3.6.2.1 Contribution of NO to vascular responsiveness

The contribution of NO to vascular responsiveness was determined by comparing responses in control animals with those receiving an inhibitor of NOS. In each animal, the diameter of the study vessel was monitored for 30 min before the infusion of saline (1 ml/h, control group) or N^G-monomethyl-L-arginine (L-NMMA, 3 mg/kg/min, 100 mg/kg in 30 min) to block NO synthesis (L-NMMAgroup).

The agonists (bradykinin (BK) 1-100 nM; acetylcholine (ACh), 10 nM-10 μM) were diluted into Krebs buffer in appropriate concentrations and were delivered in the superfusate as described for the ADO experiments. Arteriolar diameters were normalized to the baseline diameter before each drug administration, and responses to each drug were expressed as a percentage of the corresponding baseline diameter. Dose-response curves were constructed using averaged responses for individual agonists. The average peak responses to ACh and adenosine occurred at 90 s, whereas the average peak response to BK occurred at 120 s. Data were obtained using either ketamine, halothane or isoflurane anesthesia.

3.6.2.2 Contribution of EDHF to vascular responsiveness.

In the present study, superfusion of the cremaster muscle with high [K^+] buffer was used to inhibit the action of EDHF. For these studies, responses to agonists were obtained under normal [K^+] (5 mM) and then during superfusion with high [K^+]. High [K^+] buffer was superfused for 30 min before agonist responses were re-tested. Agonists were administered as described above.

3.6.3 Studying the role of K_{ATP} channels in the levosimendan- and OR-1896- induced vasodilatation

We used pentobarbital anesthesia in these experiments. The arteriolar responses to acetylcholine (1 μM) and adenosine (10 μM) were then recorded to assess the viability of the preparation. Blood vessel diameters were reported as absolute values in μm in this study. The

blood vessel dimeters were continuously recorded and data obtained at the peak of the drug-induced dilatation are reported.

3.6.3.1 Levosimendan- and OR-1896-induced vasodilatation responses

The vasodilatation induced by levosimendan and its active metabolite, OR-1896, were studied in the resistance arterioles of the cremaster muscle. Cumulative concentrations of levosimendan (1 nM – 100 μM) or OR-1896 (1 nM – 100 μM) were administered (100 μl in a bolus) topically to the surface of the cremaster muscle and the changes in arteriolar diameter were recorded.

3.6.3.2 Investigating the role of K_{ATP} channels levosimendan- and OR-1896- induced vasodilatation

First, the arteriolar responses to cumulative concentrations of the K_{ATP} channel opener pinacidil (1 nM – 10 μM) were recorded in the absence or presence of glibenclamide (5 μM, in the superfusion solution for 30 min), a selective inhibitor of K_{ATP} channels. This was followed by observation of vascular diameter changes in response to levosimendan (1 nM – 100 μM) or OR-1896 (1 nM – 100 μM) in the absence or presence of glibenclamide

Stock solutions of levosimendan, OR-1896, pinacidil and glibenclamid were prepared in dimethyl-sulfoxid (100 mM DMSO stock), and further diluted in ethanol. Test solutions from stock were prepared freshly on the day of the experiments.

3.7 Materials

All chemicals were obtained from SigmaChemical (St. Louis, MO, USA) except the followings: halothane and ketamine from Halocarbon Laboratories (River Edge, NJ, USA), isoflurane and pancuronium bromide from Gensia Pharmaceuticals (Irvine, CA, USA), adenosin from Research Biochem. Inc (Natick, MA, USA), indometacin, acetylcholin and bradykinin from Cyclops Biochemicals (Salt Lake City, UT, USA) L-NMMA from Parke Davis (Morris Plains, NJ, USA). Levosimendan and OR-1896 are the products of Orion Pharma (Espoo, Finland).

3.8 Data analysis and statistical tools

In the first set of the experiments average blood vessel diameters were recorded between 5-10 min following the start of the superfusion with solutions having various $[K^+]$. All data are reported as means±SEM from the indicated number of experiments. Repeated measures

ANOVA and Dunnett's test were used to evaluate the effects of increasing [K^+] or adenosine on arteriolar diameters. Paired t tests were used to compare responses before and after treatment in the same animal. $P < 0.05$ was considered statistically significant.

In the second set of experiments arteriolar diameters were normalized to the baseline diameter before each drug administration, and responses to each drug were expressed as a percentage of the corresponding baseline diameter. Dose-response curves were constructed using averaged responses for individual agonists. Data were presented as means ± SEM from the indicated number of experiments. The group means were compared using the Student's t-test for unpaired data. Multiple comparisons among more than two groups were analyzed by analysis of variance followed by Student-Newman-Keuls (SNK) test. Differences were considered significant at $P < 0.05$.

In the third set of experiments drug-induced arteriolar diameter changes are presented in absolute values. Vascular diameter changes were recorded continuously, and peak arteriolar responses were selected to illustrate the drug-induced effects. Data are expressed as the means±SEM from the indicated number of experiments. Statistical analyses were performed with two-way repeated-measures ANOVA, followed by Bonferroni's post-hoc test. Differences were considered significant at $P < 0.05$.

4 Results

4.1 Involvement of K^+ channels in the regulation of blood vessel diameter at normal extracellular [K^+]

Cremaster muscle arteriolar diameters were observed during superfusion with buffer containing 5 mM K^+ in the absence (control) and presence of K^+ channel inhibitors in paired comparison. The following ion channel inhibitors were used: barium ($BaCl_2$, 50 mM), glibenclamide (GLIB, 1 μM), or iberiotoxin (IbTX, 100 nM) to block K_{IR}, K_{ATP}, or K_{Ca} channels (K_{Ca} 1.1), respectively.

Under control conditions (5 mM K^+, no inhibitors), vessel diameters were significantly decreased by GLIB and Ba^{2+}, but not by IbTX, suggesting that basal diameter was regulated by ATP-sensitive and inward-rectifier K^+ channels. Ba^{2+}, glibenclamide, or IbTX did not influence the vasodilator response to adenosine (ADO). This way ADO was used as an easily applicable positive control to test vascular responsiveness.

4.2 Involvement of K^+ channels in the vasodilation response at modestly increased extracellular [K^+]

Of the ion channel inhibitors applied in our study only extracellular Ba^{2+}, but not GLIB or IbTX prevented increased [K^+]-induced dilation of the arterioles. The data indicate that the inward-rectifier K^+ channel (blocked by low concentrations of Ba^{2+}, but not GLIB or IBTX) was most likely responsible for the K^+- induced arteriolar dilation in fourth order arterioles of the rat cremaster muscle.

These experiments directed us to the conclusion that under physiological condition both $K_{IR,}$ and K_{ATP} channels participate in the maintenance of the baseline arterial tone in fourth order arterioles of the rat cremaster muscle. In addition, pharmacological inhibition of K_{IR} channels prevents the modestly high extracellular [K^+]-induced dilation of the arterioles.

4.3 The role of various anesthetic agents in the regulation of vascular tone

Experiments shown in the previous sections of the Results were performed *in vivo*. This required anesthesia of the experimental animals. To elucidate if the method of anesthesia influences microcirculation we characterized the baseline vessel diameters in the absence of agonists and the vasodilation-response induced by two well-characterized agonists (acetylcholine, ACh and bradykinin, BK) in animals anesthetized using different drugs. As a first step we determined if various anesthetic agents influence the NO- and EDHF-dependent regulatory processes in the absence of an agonist (i.e. baseline regulation of vascular tone).

We used either L-NMMA to inhibit NOS or increased extracellular [K^+] (20 mM) to inhibit EDHF action or both to block NOS and EDHF simultaneously. Thus, results were obtained in four treatment groups: 5 mM [K^+] (control); 5 mM [K^+] + L-NMMA; 20 mM [K^+]; and 20 mM [K^+] + L-NMMA. The vasodilation mechanisms remaining available after the treatments in each treatment group were, NO+EDHF-mediated, EDHF-mediated, NO-mediated, or neither EDHF nor NO-mediated, respectively.

All rats were pretreated with indomethacin (5 mg/kg iv), this dose of indomethacin has been demonstrated to block the synthesis of prostaglandin in rats.

4.3.1 Effect of the inhibition of the NOS pathway on the vessel diameter as a function of the anesthetic agent used.

All studies were performed using rats anesthetized with either ketamine, halothane or isoflurane. After the surgical procedure we applied indomethacin to block prostaglandin synthesis, and the muscle was superfused by warmed Krebs buffer (5 mM [K^+]). The preparation was allowed to equilibrate for at least 30 min before the start of the experimental protocol. We did not study the same vessel before and after L-NMMA because the time required to complete the entire protocol would have been longer than we have shown the preparation to be stable. In each animal, the diameter of the study vessel was monitored for 30 min before and after the intravenous infusion of saline (1 ml/h, control group) or L-NMMA (3 mg /kg / min). After 30 mins of L-NMMA or saline infusion (altogether 60 mins after surgical preparation) intraarterial systolic blood pressure and basal vessel diameter was recorded. First we compared the influence of the anesthetic agents on blood pressure and basal vessel tone.

Mean arterial blood pressures during halothane anesthesia were significantly lower (80±3 mmHg, n=14) than during either isoflurane (96±2 mmHg, n=12) or ketamine (98±3 mmHg, n=17) anesthesia, as observed previously (ANOVA and SNK, $P<0.001$). Inhibition of NOS with LNMMA significantly increased mean arterial blood pressure to 118±4 mmHg (halothane, n=12), 152±2 mmHg (isoflurane, n = 13), or 146±4 mmHg (ketamine, n = 14) in all groups, (t-test, $P<0.001$ in each groups).

Baseline fourth-order arteriolar diameters tended to be greater during anesthesia with isoflurane

(25 ± 1 μm, n=13), then during halothane (21±1 μm, n=16) or ketamine (20±2 μm, n=17) anesthesia, but the difference did not reach statistical significance ($P = 0.1$, ANOVA). ADO (100 μM), a known endothelium – independent vasodilator was applied to test vessel responsiveness.

Arteriolar diameters in rats treated with L-NMMA during anesthesia with isoflurane (25 ± 1 μm, n=14), halothane (19±2 μm, n=14), or ketamine (20±2 μm, n=15) were not different from the diameters in control rats (see above), t-test, $P>0.3$ in each groups.

4.3.2 Effect of the inhibition of the EDHF pathway on the vessel diameter as a function of the anesthetic agent used.

In the present study, superfusion of the cremaster muscle with high [K^+] buffer was used to inhibit the action of EDHF. First we examined the possible systemic effects of high K^+ buffer administration over the muscle surface. Superfusion of the cremaster muscle with 20 mM [K^+] did not significantly influence systemic plasma K^+ concentration (in 4 rats, 3.8 ± 0.21 mmol/l before and 4.65 ± 0.45 mmol/l after 3 h of superfusion with 20 mM [K^+]) or systemic blood pressure.

Superfusion of the cremaster muscle with high [K^+] buffer (20 mM) to inhibit EDHF responses significantly dilated cremaster muscle arterioles during halothane, isoflurane, or ketamine anesthesia compared with the diameters observed in 5 mM K^+. The vasodilator effect of high K^+ was not altered by L-NMMA treatment.

Next we recorded the response of the vessels to 100 μM adenosine, which causes vasodilation in an endothelium-independent mechanism. It was not impaired by L-NMMA during anesthesia with any of the anesthetics, demonstrating that the response was NO independent. When expressed as a percentage of the baseline diameter, the vasodilator response to adenosine under control conditions (5 mM K^+) was similar during anesthesia with isoflurane (187 ± 21%, n=13), halothane (179 ± 10%, n=15), or ketamine (189 ± 14%, n=15). The response to adenosine was unaffected by L-NMMA treatment during anesthesia with isoflurane (180 ± 15%, n=14), or ketamine (206 ± 15%, n=17). We concluded from these that the response of the blood vessel is mediated by a NO-independent pathway. However, L-NMMA treatment significantly enhanced the response to adenosine during halothane anesthesia (248 ± 13%, n=16).

In the next set of experiments we tested the ADO-induced vasodilation response at high (20 mM) extracellular [K^+]. We found that the vasodilator response to adenosine was not reduced by high [K^+] buffer alone or high [K^+] buffer combined with L-NMMA administration. The peak diameter reached was approximately equal (35-40 μm) in all anesthetic groups in the presence of normal or high [K^+].

4.3.3 Acetylcholine-induced vascular responses in the presence of various anesthetic agents

The acetylcholine-induced vasodilatation responses were compared under isoflurane, halothane and ketamine anesthesia. The contribution of various pathways leading to

vasodilation were assessed using L-NMMA or high [K^+] superfusion to inhibit the NO-dependent and EDHF-dependent pathways, respectively. The ACh-induced vasodilation responses were similar in magnitude and kinetics to those evoked by ADO under physiological conditions (5 mM [K^+], no pharmacological agents).

We found that the ACh-induced vasodilation can be significantly reduced by L-NMMA, the competitive inhibitor of eNOS, at normal (5 mM) [K^+] in the superfusate. Thus, we demonstrated that the vasodilation response induced by ACh includes a NO-dependent mechanism in the resistance arterioles of our model system.

On the contrary, the inhibition of the EDHF mechanisms using high [K^+] in the superfusate did not influence, in principle, the ACh-induced vascular responses. When L-NMMA was combined with 20 mM [K^+] in the superfusate a reduction of the vasodilation was observed in the presence of 1 μM ACh. This effect, however, was not reproducible at other agonist concentrations. Therefore, we regarded this phenomenon non-significant and biologically irrelevant. Of note, we expressed the agonist-induced vasodilation as a % of initial vessel diameter. As vessel diameters were significantly larger at high (20 mM) [K^+] in the superfusate the corresponding agonist-induced vascular responses were reported smaller than those observed in 5 mM [K^+].

As opposed to the observations during isoflurane anesthesia, rats anesthetized using halothane or ketamine did not display NO-dependent components of the ACh-induced vasodilation either under physiological conditions (5 mM) or high [K^+] (20 mM).

In summary, our results indicate that the choice of the anesthetic agent influenced the mechanism responsible for ACh-induced vasodilation. The process is NO-dependent at 5 mM [K^+] in the superfusate whereas NO-independent mechanisms are responsible for the vasodilation under halothane and ketamine anesthesia.

4.3.4 Bradykinin-induced vascular responses in the presence of various anesthetic agents

In the next set of experiments the bradykinin-induced vasodilatation responses were compared under isoflurane, halothane and ketamine anesthesia. The contribution of various pathways leading to vasodilation were assessed using L-NMMA or high [K^+] superfusion, as discussed in the previous chapters. The BK-induced vasodilation responses were similar in magnitude and kinetics to those evoked by ADO under physiological conditions (5 mM [K^+], no pharmacological agents).

We found that BK-induced (1-10nM) vasodilation can be significantly reduced by L-NMMA, the competitive inhibitor of eNOS, at normal (5 mM) [K^+] in the superfusate. Thus, we demonstrated that the vasodilation response induced by BK includes a NO-dependent mechanism in the resistance arterioles of our model system, like the ACh-induced vasodilation does. We found a short-lived (60 s, 90 s) vasodilation response to BK at one concentration (10 nM) only when the EDHF pathway was inhibited using 20 mM [K^+] in the superfusate.

As opposed to the observations during isoflurane anesthesia, rats anesthetized using halothane or ketamine did not display consistent NO-dependent components of the BK-induced vasodilation either at physiological (5 mM) or high (20 mM) [K^+].

In summary, our results indicate that the choice of the anesthetic agent influenced the mechanism responsible for BK-induced vasodilation when the EDHF pathway is intact (i.e., at 5 mM [K^+] in the superfusate). Thus, vasoregulatory responses and signaling pathways of resistance arterioles in the microcirculation are sensitive to the anesthetic agents applied.

Both ACh and BK evoke NO-dependent vasodilation responses under isoflurane anesthesia at physiological [K^+] in the superfusate since inhibition of the NOS system resulted in a diminished vasodilation response to these agonists. Based on these experiments we conclude that the balance between the NO-dependent and EDHF-dependent regulation of the vascular tone is impaired under isoflurane anesthesia: the contribution of the NO-dependent pathway increases whereas the that of the EDHF-pathway is diminished in the regulation of the vascular tone of resistance arterioles.

Neither ACh-dependent nor BK-dependent vasodilation was sensitive to the inhibition of the NOS and/or the EDHF pathway under halothane and ketamine anesthesia.

4.4 The vasoactive propertioes of levosimendan and its active metabolite, OR-1896, *in vivo*

During a 30-min incubation period a spontaneous tone developed in the cremaster muscle arterioles (resting diameter of 25 ± 6 μm). The endothelium-dependent vasodilator acetylcholine (1 μM) and the endothelium-independent agonist adenosine (10 μM) elicited substantial dilations in the cremaster muscle arterioles (dilations from 25±2 to 33±3μm and from 24±4 to 35±2μm, respectively), indicating the viability of the endothelium and smooth muscle cells in the preparations and that both the endothelium-dependent and endothelium-

independent pathways are intact in the cremaster muscle resistance arterioles. This also means that both the endothelium cells and the smooth muscle cells are intact and respond properly to the stimuli.

4.4.1 Vasodilation responses evoked by levosimendan and OR-1896

The responses of the cremaster muscle arterioles to increasing concentrations of levosimendan or of OR-1896 were then recorded. The topical administration of levosimendan induced concentration-dependent (1 nM – 100 μM) arteriolar dilation (maximal dilation from 23 ± 2 to 33 ± 2 μm). The application of OR-1896 (1 nM – 10 μM) likewise induced dilation in the cremaster muscle arterioles (maximal dilation from 22 ± 1 to 32 ± 1 μm).

The magnitudes of the dilations induced by levosimendan or by OR-1896 were similar at all investigated concentrations ($P>0.05$).

As described in the Materials and Methods section both levosimendan and OR-1896 were dissolved in dimethyl-sulfoxid (DMSO, 100 mM stock solution) which was diluted further in ethanol prior to the final dilution in the superfusion buffer. The maximum concentration of the solvent vehicle DMSO was 0.015% in the superfusion solution, a concentration that had no significant effect on arteriolar diameter.

4.4.2 Role of K_{ATP} channels in mediating levosimendan and OR-1896-induced arteriolar dilations

The changes in cremaster muscle arteriolar diameter in response to the K_{ATP} channel opener pinacidil (1 nM – 10 μM) were first recorded in the absence or presence of glibenclamide (5 μM, 30 min), a selective blocker of K_{ATP} channels. Incubation with glibenclamide alone had no effect on the basal arteriolar tone (the diameters were 23 ± 2 and 23 ± 2 μm before and after the incubation with glibenclamide). However, in the presence of glibenclamide, the dilations in the cremaster arteriole in response to pinacidil were found to be completely abolished.

Next, the potential involvement of K_{ATP} channels in levosimendan- or OR-1896-induced dilation was investigated. Similarly to the findings with pinacidil, the dilation in response to levosimendan was diminished by glibenclamide (maximal dilation to 23 ± 3 μm). The OR-1896-induced dilation was also opposed by glibenclamide (maximal dilation to 22 ± 5 μm). The vascular diameters at the beginning of the experiments (initial diameters) and those at

maximal levosimendan or OR-1896 concentration did not differ significantly in the presence of 5 μM glibenclamide.

Our experiments showed that levosimendan and its active metabolite, OR-1896, evoke significant and very effective vasodilation in the arterioles of the rat cremaster muscle *in vivo*. We have provided pharmacological evidence for the key role of drug-induced activation of the K_{ATP} channels in this response. The levosimendan- and OR-1896-evoked responses were similar in magnitude and dose-response, thus, we propose that the active metabolite and the parent molecule are equally potent vasodilators.

5 Discussion

5.1 The K_{IR} channel contributes to the vasodilation induced by modest increase in the $[K^+]$ of the superfusate. The K_{ATP} channel also contributes to the maintenance of the basal vascular tone.

Modest increase in the $[K^+]$ of the superfusate (10mM) resulted in vasodilation in the model system used in our experiments. The mechanism of action of the increased $[K^+]$ is likely to be the activation of the K_{IR} channel in the vascular smooth muscle cells: increased $[K^+]$-induced vasodilation could be inhibited by Ba^{2+}, the blocker of the K_{IR} channels, but was insensitive to iberiotoxin and glibenclamide, the blockers of K_{Ca} and K_{ATP} channels, respectively. These results are in good agreement with the literature. Edwards and co-workers showed in hepatic and mesenteric arteries of the rat that the vasodilation induced by increase in the extracellular $[K^+]$ is mediated by K_{IR} channels. Our experiments provided functional evidence for the existence of K_{IR} channels in the arterioles of the cremaster muscle in rats. We also showed that K_{IR} channels can be activated *in vivo* by a modest increase in the extracellular K^+ concentration and this might, at least partially, explain the $[K^+]\uparrow$-evoked vasodilation response. To inhibit K_{IR} we used Ba^{2+}, which, depending on its concentration, may interact with other ion channels as well (e.g. a K_{ATP}, K_{Ca} and K_v). On the other hand, we used a very small Ba^{2+} concentration at which the divalent cation is considered to be specific for K_{IR} channels. Under physiological conditions the excess K^+ required for vasodilation may originate from the skeletal muscle cells firing rapidly action potentials. Stekiel et al. showed that potassium-induced dilations of rat cremaster muscle arterioles were not due to local activation of peripheral nerves, since the response was not blocked by treatment with 6-hydroxydopamine, to destroy sympathetic nerve terminals, or the inclusion of TTX,

phentolamine, and propranolol in the muscle superfusate to inhibit neuronal activation and catecholamine action.

An additional finding of this study was that in addition to the K_{IR} channels, K_{ATP} channels are also important regulators of vascular tone under physiological ionic conditions (5 mM K^+) *in vivo.* The strongest support of this finding is that application glibenclamide caused significant constriction of fourth order arterioles of the rat cremaster muscle. This has been reported previously in hamster arterioles. Our data demonstrate that K_{ATP} channels regulate active tone in rat skeletal muscles as well. Application of the charybdotoxin, a high affinity inhibitor of K_{Ca} channels decreases the resting diameter of isolated cerebral, coronary and, mesenteric resistance arteries. Thus, K_{Ca} channels seem to be important for the regulation of vascular tone in these arteries. Nevertheless, the sensitivity of the vascular tone to charybdotoxin was not seen by us or by others in rat muscle- which indicates K_{Ca} channels do not participate in the vasodilation response of rat muscle arterioles.

In summary we have shown that both K_{IR} and a K_{ATP} channels play a pivotal role in the maintenance and regulation of resting vascular tone in striated muscle of rats *in vivo*. Our data provided the first functional evidence for the existence of K_{IR} channels in the fourth order arterioles of the cremaster muscle of rats. We also showed that K_{IR} channels contribute to the vasodilation response in these arterioles induced by modest increase in the extracellular K^+ concentration.

5.2 The balance of NO and EDHF is altered during anesthesia pending on the choice of the anesthetic agent

Data in this dissertation also showed the effect of different volatile anesthetics on the regulation of microcirculation in the fourth order arterioles of the cremaster muscle of rats *in vivo*. We hypothesized that the various anesthetic agents applied will influence the role of NO and EDHF in the ACh- and BK-induced vasodilation. The NO pathway was inhibited by L-NMMA whereas the action of EDHF was inhibited by the application of high (20 mM) [K^+] in the superfustate. We applied indomethacin in all experiments to suspend the prostaglandin-dependent pathway of vasoregulation.

Inhibition of the NO and EDHF pathway separately allowed us to determine the relative contributions on these pathways to agonist-induced vasodilation *in vivo* under halothane, isoflurane or ketamine anesthesia.

Systemic, intravenous application of L-NMMA resulted in a significant increase in the systolic blood pressure regardless of the choice of the anesthetic agent. This data points to an active NO system in the regulation of vessel diameters and that of peripheral resistance. In addition, agonist-induced vasodilation was diminished when the NO pathway was inhibited during isoflurane anesthesia only, but not when animals were anesthetized using halothane or ketamine. We do not precisely understand this phenomenon, it may well be that arterioles of other organs or vessel at other level of branching would have behaved differently.

We do not understand either why halothane increases the vascular responses to adenosine when the NO pathway was inhibited using L-NMMA. Furthermore, this effect was only seen when the K^+ concentration of the superfusate was 5 mM, but not at high K^+ concentration.

Apart from the still open questions, we think that our results are very important regarding the design of *in vivo* microcirculation experiments: we showed that the choice of the anesthetic agent may influence the balance of the NO-dependent and NO-independent mechanisms of vasodilation. Inhibition of the NOS pathway diminished the vasodilation responses to ACh and BK during isoflurane anesthesia. Based on this we concluded that NO-dependent mechanisms are enhanced during isoflurane anesthesia. Under identical conditions neither ACh- nor BK-dependent vasodilation was sensitive to NOS inhibition when rats were anesthetized using halothane or ketamine.

5.3 Levosimendan and its active metabolite induce vasodilation in peripheral arterioles by activating K_{ATP} channels

5.3.1 Levosimendan and OR-1896 are strong vasodilators in vivo

The present study has revealed pronounced vasodilator potentials for both levosimendan and its long-lived positive inotropic metabolite OR-1896 in the rat skeletal muscle microcirculation, through K_{ATP} channel activation *in vivo*.

It was shown in the last few years that the beneficial hemodynamic effects of levosimendan and OR-1896 can only be partially attributed to their calcium-sensitizing effect. Similar to that of levosimendan, OR-1896 exerts a potent vasodilatory effect on isolated coronary and skeletal muscle arteries in the rat. Both levosimendan and OR-1896 have been demonstrated to act as vasodilators ex vivo in arteries and veins with diameters larger than 100 μm. Moreover, indirect microvascular effects of levosimendan and OR-1896 in whole animals have been investigated through measurement of the changes in systemic vascular resistance

following systemic drug infusion, whereas no experimental evidence has been provided for their direct effects on the microcirculation and resistance-sized arterioles of 20-25 μm diameter *in vivo*.

In the present study, we monitored the effects of levosimendan and OR-1896 on the arteriolar responses (fourth order arterioles of 20-25 μm diameter) directly by means of intravital microscopy on the rat cremaster muscle. Our results demonstrated that both levosimendan and OR-1896 may decrease the vascular resistance in vessels *in vivo* which are not antagonized by cardiovascular reflex regulation.

The maximal vasodilation achieved in response to levosimendan or OR-1896 were similar at all concentrations investigated. Based on this it is reasonable to propose that the reason why the metabolite and its parent compound are equipotent vasodilators may be that the mode of action of the two molecules is similar.

It should be noted that levosimendan and OR-1896 also induced significant vasodilation at the therapeutically relevant concentration of 10 nM. These *in vivo* features of levosimendan and OR-1896 were reminiscent of those observed *in vitro* by others.

5.3.2 The vasodilation induced by levosimendan and OR-1896 is caused predominantly by K_{ATP} channel activation

We currently attempted to elucidate the relative contribution of K_{ATP} channel activation in levosimendan- and OR-1896-induced vasodilation by using K_{ATP} channel function modulators in the cremaster arteriole of the rat *in vivo*. Both levosimendan- and OR-1896-induced arteriolar dilations proved to be effectively diminished by the selective K_{ATP} channel blocker glibenclamide, similarly to when the K_{ATP} channel opener pinacidil was combined with glibenclamide. Glibenclamide practically abolished levosimendan or OR-1896-induced vasodilation, implicating K_{ATP} channels as the most significant mediators of these induced microcirculatory responses. Nevertheless, I would like to point out that the above regulatory effect was demonstrated in male animals in this study, and hence, the involvement of K_{ATP} channels in vasodilation might be affected by gender to some degree.

At the moment, we do not have a clear-cut explanation for the complexity of levosimendan-induced vascular responses in various vessels and species, and we therefore speculate that the expressions and/or regulations of K_{ATP} and BK_{Ca} channels are not uniform in the investigated vascular preparations.

The present results do not support the hypothesis of additional vasodilator mechanisms involving partial mediation of the action of levosimendan and OR-1896 by cAMP. In fact, these two molecules were equipotent vasodilators *in vitro* and *in vivo*; both could be antagonized by glibenclamide and, as indicated by a previous study of our group, displayed profound differences as phosphodiesterase inhibitors.

Of note, there is an apparent controversy between the first set of experiments and this current set regarding the effect of glibenclamide. We have shown previously that at 5 mM [K^+] of the superfusate glibenclamide decreased the basal tone of the resistance arterioles, i.e., K_{ATP} channels seemed to contribute to the regulation of the basal arteriolar tone. In the second set of experiments we were unable to show this effect of glibenclamide on the resting vascular tone. Since the properties of the experimental animals (sex, weight, age) were the same and the technique used to measure vascular responses were virtually identical we propose that the difference in the method on anesthesia (halothane vs. pentobarbital) can be accounted for the different outcomes of the experiments. This hypothesis is in line with our other results presented in the thesis thereby underlining the importance of the method of anesthesia for the interpretation of the results: one or the other vasoregulatory mechanism may be dominant as a depending on of the choice of the anesthetic agent.

Nevertheless, the data reported here suggest that levosimendan and its metabolite OR-1896 act on the same molecular target in resistance arterioles of the systemic circulation by activating K_{ATP} channels and leading to substantial vasodilation of the rat cremaster muscle arteriole *in vivo*. It can be assumed that levosimendan and OR-1896 mediate effects on microvascular resistance through this molecular interaction in skeletal muscle, thereby contributing to both the acute and long-term hemodynamic effects subsequent to levosimendan administration.

5.4 Practical significance of the results

My results contribute to our current understanding of regulatory processes in the microcirculation. In addition, my results also facilitate a more global view of the mechanism of action of drugs applied in medicine. The results presented in the thesis may capacitate further experiments in the filed aiming at the development of novel therapeutic approaches.

6 SUMMARY

We studied the changes in the diameter of fourth order cremaster arterioles of anesthetised rats, using intravital videomicroscopy technique in three different sets of experiments. The various agents studied were added to the superfusate of the muscle in all experiments. At first we investigated the contribution of different types of K^+ channels to the maintenance of the resting vessel tone. At 5 mM K^+ vessel diameters were significantly decreased by glibenclamide and barium, but not by iberiotoxin, suggesting that basal diameter of the vessels was regulated by K_{IR} and K_{ATP} channels. Subsequently, we studied how barium, glibenclamide or iberiotoxin, the specific inhibitors of K_{IR}, K_{ATP} and K_{Ca} channels, respectively, influence the reaction of the blood vessels to modest increase in K^+ concentration. We found that barium prevented K^+-induced dilation only, i.e., the K_{IR} channel can be responsible for the K^+-induced vasodilation in fourth order arterioles of the rat chreamser muscle.

In the next section of our study we investigated if the choice of anesthetics (isoflurane, halothane or ketamine) alters the balance between NO and EDHF in agonist (acetylcholine, ACh or bradykinin, BK) induced vasodilation. L-NMMA, the inhibitor of NO synthase abolished the agonist induced vasodilation during isoflurane, but not halothane or ketamine anesthesia. Our data suggest that anesthetics can alter the pathway of endothel derived vasodilation in vivo, i.e., the outcome of in vivo vascular-biology experiments may depend on the choice of anestethics: NO dependent mechanisms are enhanced and EDHF action is inhibited during isoflurane anesthesia.

In the last set of experiments we investigated the in situ effects of levosimendan and its metabolite OR-1896 in vivo. We found that the two compounds induced arteriolar dilations with identical dose-response relationships. The vasodilation-response of the arterioles to pinacidil, the opener of the KATP channel, and the inhibition of this response by the selective K_{ATP} channel blocker glibenclamide demonstrated the function of this channel in regulating vascular tone. Glibenclamide counteracted the arteriolar dilation in response to levosimendan or OR-1896 and thus, the K_{ATP} channel was isolated as a target of these vasoactive compounds. In concluson, this was the first study to demonstrate that Levosimendan and OR-1896 elicit arteriolar dilation in vivo, via activation of K_{ATP} channels in real resistance vessels in the rat cremaster muscle.

7 LIST OF PUBLICATIONS

According to the recommendation of the preliminary review committee the “list of publications related to the thesis” should include publication No 4 listed under “other publications”. Am. J. Physiol. was considered a journal having 0 impact factor in 1997 due to administrative reasons. This is when Am. J. Physiol. was split into subdisciplines and thus, technically, the impact factor was 0 for this prestigious journal.

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Candidate: Ildikó Gődény
Neptun ID: B923OP
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List of publications related to the dissertation

1. **Gődény, I.**, Pollesello, P., Édes, I., Papp, Z., Bagi, Z.: Levosimendan and its metabolite OR-1896 elicit KATP channel-dependent dilation in resistance arteries in vivo.
   Pharmacol Rep. 65, 1304-1310, 2013.
   IF: 2.165
2. Loeb, A. L., **Gődény, I.**, Longnecker, D. E.: Functional Evidence for Inward-Rectifier Potassium Channels in Rat Cremaster Muscle Arterioles.
   Microvasc. Res. 59 (1), 1-6, 2000.
   DOI: http://dx.doi.org/10.1006/mvre.1999.2187
   IF: 2.016

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List of other publications

3. Nagy, L., **Gődény, I.**, Ifj., N. P. P., Leskó, Á., Balogh, L., Bánhegyi, V., Bódi, B., Csípő, T., Csongrádi, A., Fülöp, G. Á., Kovács, Á., Lódi, M., Papp, Z.: A szív pozitív inotróp támogatása a miozin-aktivátor hatású omecamtiv-mecarbil segítségével.
   Cardiol. Hung. 47 (1), 69-76, 2017.

4. Loeb, A. L., **Gődény, I.**, Longnecker, D. E.: Anesthetics alter relative contributions of NO and EDHF in rat cremaster muscle microcirculation.
   Am. J. Physiol. Heart Circ. Physiol. 272, H618-H627, 1997.
   DOI: http://dx.doi.org/10.1152/ajpheart.1997.273.2.H618

Total IF of journals (all publications): 4,181
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