Chapter 9

General Discussion
Disease-related EDHF impairment in small arteries: Underlying mechanisms and treatment strategies

For a long time the impairment of the nitric oxide (NO) system has been the focus of attention regarding endothelial dysfunction in cardiovascular and renal disease. Furthermore, endothelial dysfunction was very often regarded to be generalized to the different vascular beds. Recently, an important role of endothelium-derived hyperpolarizing factor (EDHF), and the importance of differences between endothelium-derived mediators in arteries of different vascular beds, and in arteries with different vessel size has been proposed. Therefore, the first important aim of this thesis was to investigate the endothelial alterations associated with cardiovascular and renal disease in more detail, with emphasis on EDHF and the underlying mechanisms of its impairment, and with respect to differences in arteries derived from different vascular beds.

In summary, the results presented in this thesis to treat this aim indicate an important role of an impairment of the arterial EDHF response in different animal models of cardiovascular and renal disease (Chapter 2, 3). Furthermore, the results emphasize the overall differences in the contribution of EDHF to mediate endothelium-dependent dilation between different vascular beds in healthy rats (Chapter 2, 3, 5, 6, 7, 8), and a differentially pronounced disease-related impairment in the EDHF response in different artery types (Chapter 2). This impairment seems to be more pronounced in NO-dependent artery types such as coronary arteries, as compared to artery types in which the endothelium-dependent dilation is rather independent of NO such as the mesenteric artery. Finally, the data provide important indications on the underlying mechanisms of EDHF impairment namely the interrelation of attenuated EDHF-mediated dilation with increased myogenic constriction, and the involvement of K$_{Ca}$ channels (Chapter 5). The potentiating effect of K$_{Ca}$ channel openers on the EDHF response demonstrated in Chapter 5 may provide new directions for potential treatment strategies to reverse disease-related defects in the EDHF response. The impact of the results a) to define potential underlying mechanisms of EDHF impairment in specific artery types, and b) the therapeutic potential of K$_{Ca}$ channel openers will now be discussed in more detail.

Mechanisms of disease-related EDHF impairment

The elucidation of the underlying mechanisms of disease-related EDHF impairment is hampered by the fact that the exact nature of EDHF has not yet been clarified. It is even not clear whether EDHF may indeed be a solid diffusing substance or rather an electrotonic spread of membrane potential, and it seems that different EDHF(s) may account for the endothelium-dependent L-NMMA- and indomethacin-resistant dilation in different artery types and species. Therefore, before we will develop potential models of underlying mechanisms of EDHF impairment, we first give a short overview of the different theories on the nature and working mechanism of EDHF, and we discuss the potential impact of different EDHF characteristics in different artery types (a more detailed review on the different research lines approaching the identification of EDHF in different artery types is given in Chapter 10).
Current theories on the identity and working mechanism of EDHF ²⁻⁵

Theory 1) EDHF: K⁺ that is derived from endothelial cells and induces hyperpolarization and dilation of underlying smooth muscle cells.

In summary, according to this theory, endothelial receptor stimulation with endothelium-dependent dilators such as acetylcholine (ACh), and the resulting intracellular Ca²⁺ increase, may induce opening of endothelial K_{Ca} channels leading to K⁺ efflux from endothelial cells into the myoendothelial space. This elevated (extracellular) potassium concentration may induce opening of K_{IR} channels and/or activation of Na⁺/K⁺-ATPase on underlying vascular smooth muscle cells leading to hyperpolarization, closure of voltage-sensitive Ca²⁺ channels (VSCCs) and dilation of smooth muscle cells ⁶⁻⁹ (Figure 1).

Theory 2) EDHF: electronic spread of a hyperpolarizing current via myoendothelial gap junctions from endothelial cells to smooth muscle cells.

According to this theory, receptor stimulation of endothelial cells induces hyperpolarization of endothelial cells, likely via opening of endothelial K_{Ca} channels, and the hyperpolarizing current is then propagated to smooth muscle cells via myoendothelial gap junctions leading to hyperpolarization, closure of VSCCs and dilation of smooth muscle cells ¹⁰,¹¹⁻¹⁵ (Figure 1).

Theory 3) EDHF: epoxyeicosatrienoic acids (EETs) that are produced in endothelial cells act on underlying smooth muscle cells.

According to this theory, after endothelial receptor stimulation, the eicosanoid 11,12 EET* is produced in endothelial cells by the enzyme cytochrome P450, subsequently acting on underlying smooth muscle cells where it induces opening of smooth muscle K_{Ca} channels leading to hyperpolarization, closure of VSCCs and dilation ¹⁶,¹⁷⁻²₅ (Figure 1).

What all three theories have in common is that opening of K_{Ca} channels - either localized on endothelial cells (theory 1 and 2) or on smooth muscle cells (theory 3) - is a crucial event in EDHF-mediated dilation, and this is supported by the consistent inhibition of EDHF-mediated dilation by potassium channel (K_{Ca}) inhibitors *, i.e. charybdotoxin plus apamin ²⁶⁻²⁸.

As these theories on the nature of EDHF are mostly derived from studies with arteries from different vascular beds and species, it is likely that the nature of EDHF differs according to the vascular bed and species studied. In Chapter 5 we found considerable differences between the characteristics of EDHF-mediated dilation in rat mesenteric as compared to coronary arteries, i.e. the former showing a large EDHF contribution, which was unaffected by K_{Ca} channel openers, and the latter showing much less EDHF contribution, which could strongly be enhanced by K_{Ca} channel openers. In line with this studies on the nature of EDHF revealed large differences between these two artery types. Taking together those results, it seems that in mesenteric arteries K⁺ as well as gap junctions dominate in mediating the EDHF response ⁶,¹⁴,²⁹⁻³₃, whereas in coronary arteries cytochrome P450-derived metabolites from arachidonic acid (11,12 EET) rather than K⁺ and gap junctions may play a dominant role in mediating the EDHF response ¹⁶,²⁰⁻²₃,²₅,³₄⁻₃₆. As it seems that coronary arteries are very prone to develop endothelial impairment in response to certain risk factors, as compared to other artery types, it may be tempting to speculate about a relation with its EDHF characteristics.

* More background information on this topic is given in Chapter 10
Figure 1 Scheme of the current theories on the working mechanism of EDHF. CYP450, cytochrome P450; 11,12 EET, 11,12 epoxyeicosatrienoic acid; VSCCs, voltage-sensitive Ca\(^{2+}\) channels.

Differences in EDHF characteristics – does it differentiate artery types for their susceptibility to risk factors?

Clinical studies have shown that coronary arteries are sensitive to develop endothelial impairment in response to certain risk factors \(^{37-40}\). In this respect, coronary artery disease in association with progressive renal failure has been reported, although the underlying mechanisms are largely unknown \(^{41-43}\). In support of this association, in Chapter 2, we demonstrated in an experimental rat model of renal disease that indeed coronary endothelium-dependent dilation was impaired compared to rats without renal disease, whereas endothelial function in another artery type, i.e. mesenteric arteries, was not affected. Therefore, this model seemed to us being an interesting and suitable tool to explore underlying mechanisms of coronary artery-specific alterations in endothelial function. Investigating the underlying mechanisms of coronary endothelial impairment in this model we found that the impaired endothelium-dependent dilation was due to an impaired NO activity, and in addition to a large part due to an impaired EDHF response. In contrast, the EDHF response in mesenteric arteries was not impaired but rather increased in this study. In this respect, an interesting finding has been described in NO-synthase deficient mice. Consistent with the idea that EDHF may compensate during impaired vascular production of NO \(^{35,44-47}\) it has been shown that in NO-synthase deficient mice the endothelium-dependent dilation was indeed mediated by EDHF in arteries in which the same functional response was mediated by NO in normal mice \(^{48-52}\). However, this compensation phenomenon seemed to be specific to certain vascular beds,
as it could be demonstrated, amongst others, in mesenteric arteries, but not in coronary arteries. Together these data may further support the idea that the high sensitivity of coronary arteries for endothelial impairment may (at least in part) arise from its different EDHF characteristics, i.e. a lack in the compensatory EDHF response when the NO pathway is impaired, as is the case in response to certain risk factors. What then makes the difference between the two artery types?

While further elucidating specific functional characteristics of coronary arteries as compared to mesenteric arteries in normal healthy rats, in Chapter 5 we found that coronary arteries develop considerably higher myogenic constriction in response to pressure compared to mesenteric arteries. We then questioned whether there might be a relation between the myogenic response of the smooth muscle on the one hand, and the EDHF response on the other hand. In this respect, both EDHF-mediated dilation as well as myogenic constriction involve common signaling mechanisms including the regulation of vascular ion channels to set arterial tone. Therefore, we speculated that there might be a relation between the two mechanisms potentially involving vascular ion channel regulation, and that this relation could give further direction to the mechanisms underlying disease-related EDHF impairment in coronary arteries, as well as other artery types.

**Interrelation between myogenic constriction and EDHF-mediated dilation in coronary arteries from healthy rats. Role of K\(_{Ca}\) channels**

In Chapter 5 we could show a functional relation between myogenic tone and EDHF-mediated dilation in coronary arteries from healthy rats by demonstrating that coronary arteries with individually higher levels of myogenic tone showed lower levels of EDHF-mediated dilation, and vice versa. Furthermore, elevating myogenic constriction by increasing the intraluminal pressure led to a decrease in EDHF-mediated dilation. To further elucidate the underlying mechanisms of this relation, first the specific signaling pathways of myogenic constriction and EDHF-mediated dilation might be compared to define potential points of interaction. The principal basic mechanism of myogenic constriction is that intraluminal pressure-increase induces depolarization of the vascular smooth muscle membrane, opening of voltage sensitive Ca\(^{2+}\) channels (VSCCs), intracellular Ca\(^{2+}\) increase, and contraction. Furthermore, there is no doubt about the involvement of smooth muscle K\(_{Ca}\) channels in the regulation of this process, although there do exist different theories on the exact mechanism (for details see Chapter 10). As seen in Figure 2, it has been proposed that pressure increase leads to production of the cytochrome P450-derived eicosanoid 20-HETE in smooth muscle cells, and that 20-HETE, via activation of protein kinase C (PKC), induces closure of K\(_{Ca}\) channels on smooth muscle cells, thereby leading to depolarization, opening of VSCCs, and myogenic constriction. In contrast, especially in coronary (and renal) arteries of several species, EDHF is believed to be the cytochrome P450-derived eicosanoid 11,12 EET, which induces (not closure but) opening of K\(_{Ca}\) channels on smooth muscle cells, thereby leading to hyperpolarization, closure of VSCCs and dilation. It becomes obvious that there are several points where both mechanisms may compete, e.g. at the level of closing/opening of smooth muscle K\(_{Ca}\) channels, and thereby at the level of smooth muscle depolarization/hyperpolarization, and at the level of opening/closing of smooth muscle VSCCs, respectively.

* More background information on this topic is given in Chapter 10
Figure 2 Proposed model of interaction between myogenic constriction and EDHF-mediated dilation in coronary arteries. CYP450, cytochrome P450; 11,12 EET, 11,12 epoxyeicosatrienoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; PKC, protein kinase C; VSCCs, voltage-sensitive Ca\(^{2+}\) channels.

To experimentally test the involvement of K\(_{Ca}\) channels and VSCCs in the described relation, we investigated, in Chapter 5, the acute effect of a K\(_{Ca}\) channel opener, i.e. the potassium channel opener NS1619, as compared to other types of K\(^{+}\) channel openers, and we tested the acute effect of an VSCC inhibitor, i.e. nifedipine, on myogenic tone and EDHF-mediated dilation. We found that all channel modulators used, i.e. K\(^{+}\) channel opening as well as VSCC closure, attenuated myogenic constriction. Importantly however, this study demonstrated for the first time that only the K\(_{Ca}\) channel opener strongly increased EDHF-mediated dilation in coronary arteries, whereas it had no effect in mesenteric arteries. These results may support the following proposed model of interaction. As demonstrated in Figure 2, under conditions of increased pressure-induced myogenic constriction increased amounts of 20-HETE and/or protein kinase C may be produced in coronary smooth muscle cells, thereby reducing the open probability of smooth muscle K\(_{Ca}\) channels. In case of endothelial receptor stimulation inducing the EDHF response, the produced EDHF, i.e. 11,12 EET, exerting its action by opening smooth muscle K\(_{Ca}\) channels, may now act on K\(_{Ca}\) channels with decreased open state probability. Therefore, higher amounts of EDHF, i.e. 11,12 EET, may be needed to exert the same hyperpolarizing and dilative action on smooth muscle cells. This may be compatible

* More background information on this topic is given in Chapter 10
constriction, and may thereby reduce the ability of receptor-induced EDHF-mediated dilation. Importantly, acute treatment with a $K_{Ca}$ channel opener in this situation could then shift/restore the altered balance, and may increase the ability of receptor-induced EDHF-mediated dilation (Figure 3).

**Figure 3** Proposed model of a potential therapeutic action of $K_{Ca}$ channel openers by facilitating EDHF-mediated dilation. 11,12 EET, 11,12 epoxyeicosatrienoic acid; 20-HETE, 20-hydroxyeicosatetraenoic acid; PKC, protein kinase C.
with our findings showing a gradual decrease in EDHF-mediated dilation with increasing myogenic tone (Chapter 5). The application of the $K_{Ca}$ channel opener in our study may then have increased the open state probability of smooth muscle $K_{Ca}$ channels, and in case of endothelial receptor stimulation, the produced EDHF, i.e. 11,12 EET, may have induced opening of smooth muscle $K_{Ca}$ channels more easily to exert hyperpolarization and dilation of smooth muscle cells. Although the results of the present thesis seem to be compatible with this model of interaction, there are also several limitations which should be mentioned. The production of 20-HETE (in response to pressure) has been shown in the smooth muscle of several artery types, but not yet in rat coronary arteries. Similarly, the identity of EDHF being 11,12 EET in rat coronary arteries has also not yet definitively been proven. In case EDHF is not 11,12 EET acting on (iberiotoxin-sensitive large conductance) smooth muscle $K_{Ca}$ channels, i.e. BK$_{Ca}$ channels, then according to EDHF theory 1+2 (see above) other types of $K_{Ca}$ channels may be involved in EDHF-mediated dilation, i.e. non iberiotoxin-sensitive IK$_{Ca}$ and SK$_{Ca}$ channels, localized on endothelial cells, and these channels are likely to be different from the ones involved in myogenic tone. Then the model of interaction has to be modified. In this respect the use of specific $K_{Ca}$ channel openers which activate IK$_{Ca}$/SK$_{Ca}$ channels on endothelial cells but not iberiotoxin-sensitive BK$_{Ca}$ channels on smooth muscle cells, such as the potassium channel opener 1-EBIO, or the use of DHS-1 which seems to be specific for BK$_{Ca}$ channels on smooth muscle cells, may be an elegant approach to further differentiate between the involved channels. Importantly however, independent of the underlying mechanism, according to our results a high myogenic constriction relates to attenuated EDHF-mediated dilation, and acute treatment with a $K_{Ca}$ channel opener strongly increases EDHF-mediated dilation under this condition. Based on these findings obtained in coronary arteries from normal healthy rats it may now be interesting to elucidate how this relates to myogenic alterations and EDHF impairment in cardiovascular disease.

The potential impact of increased myogenic constriction on the EDHF response in cardiovascular and renal disease

In Chapter 4 we demonstrated that myogenic constriction is considerably increased in an experimental rat model of chronic heart failure (CHF) in small mesenteric arteries. Importantly, the increased myogenic constriction in our study was fully reversed specifically by acute blockade of angiotensin II type 1 (AT$_{1}$) receptors, suggesting a supportive role of constrictive hormones such as Ang II and/or their receptors for the increase in myogenic constriction. In this respect, not only pressure but also Ang II has been demonstrated to induce production of 20-HETE and protein kinase C in smooth muscle cells, thereby inducing closure of $K_{Ca}$ channels, and potentially reinforcing the myogenic response. It is important to mention in this respect that subthreshold concentrations of Ang II have previously been demonstrated to reinforce (renal) myogenic constriction via a protein kinase C dependent mechanism in vitro. For our proposed model of interaction between myogenic constriction and EDHF-mediated dilation this could mean the following. As illustrated in Figure 3 in cardiovascular disease increased plasma and/or tissue levels of constrictive hormones which induce production of protein kinase C and 20-HETE, such as Ang II, may reinforce the development of myogenic constriction. This may lead to a shift in the balance between myogenic constriction and EDHF-mediated dilation towards higher myogenic
That indeed the EDHF response is impaired in this model of CHF we (Chapter 3) and others have demonstrated for several artery types, although data on coronary arteries and mesenteric arteries are still lacking or are inconsistent. Furthermore we found that the level of EDHF impairment was related to the severity of cardiac damage (i.e. infarct size).

The presence of increased arterial myogenic constriction on one hand, and impaired EDHF-mediated dilation/hyperpolarization on the other hand have been described also in several other forms of cardiovascular and renal disease and for different artery types. For example, in a rat model of streptozotocin-induced diabetes increased myogenic constriction, associated with an upregulation of protein kinase C, and an impairment in the EDHF response have been shown. Similarly, in mesenteric arteries from spontaneously hypertensive rats (SHR) an increased myogenic constriction as well as impaired EDHF-mediated dilation/hyperpolarization have been found. Furthermore, some of these studies give indications for alterations in arterial membrane potential towards a more depolarized smooth muscle membrane potential, with decreased K⁺ currents, and increased Ca²⁺ influx through VSCCs in these diseases, although overall, the results on membrane potential changes, as well as on alterations in the expression number and/or function of several types of K⁺ channels in these diseases do not provide a consistent picture (as reviewed elsewhere).

First indications for the involvement of CYP450-derived 20-HETE in mediating the increased myogenic response in hypertension can be derived from studies demonstrating that CYP450 blockade, the application of CYP4A1 antisense oligonucleotides, as well as inhibition of production of 20-HETE with DDMS decreases the myogenic response in arteries from hypertensive rats, as well as on alterations in the expression number and/or function of several types of K⁺ channels in these diseases do not provide a consistent picture (as reviewed elsewhere). First indications for the involvement of CYP450-derived 20-HETE in mediating the increased myogenic response in hypertension can be derived from studies demonstrating that CYP450 blockade, the application of CYP4A1 antisense oligonucleotides, as well as inhibition of production of 20-HETE with DDMS decreases the myogenic response in arteries from hypertensive rats.

Furthermore, an upregulation of CYP expression and EET generation in hypertension, during salt loading, and in hypercholesteremia has been shown. However, thus far these studies on myogenic tone (and 20-HETE) on one hand and EDHF-mediated dilation (and 11,12 EETs) on the other hand have been performed separately. To confirm the proposed model of interaction in cardiovascular disease, there may still be the need to investigate those alterations in one integrated study.

In summary, based on our findings from Chapter 2-5 together with data derived from others it can be proposed:

- that disease-related increase of plasma and/or tissue levels of vasoconstrictive hormones (such as Ang II) may potentiate the pressure-induced myogenic response via protein kinase C and/or 20-HETE in small arteries
- that this disease-related increase in myogenic constriction may (at least in part) cause the disease-related impairment in the EDHF response due to competitive interactions at the level of KCa channels
- that accordingly, the finding in coronary arteries to develop higher levels of myogenic constriction in response to the same intraluminal pressure as compared to other artery types (already in healthy individuals) may contribute to explain the increased sensitivity for disease-related EDHF impairment in this artery type
- that KCa channel openers have the ability to facilitate EDHF-mediated dilation under these conditions

In this respect, the findings from Chapter 5 are the first to demonstrate that EDHF-mediated dilation in coronary arteries can be strongly enhanced by an KCa channel opening drug, and given the described arterial alterations in many forms of disease, and the impact of these
alterations for further disease progression, this finding may provide an important direction for future therapeutic strategies. This will now be discussed more in detail.

**Future directions on the therapeutic potential of K\textsubscript{Ca} channel openers**

Independent of the exact mechanisms underlying the enhancing effect of K\textsubscript{Ca} channel opening on EDHF-mediated dilation found in Chapter 5, this effect may have interesting therapeutic implications. The results demonstrate an obvious vascular-bed specific facilitation of EDHF-mediated dilation by K\textsubscript{Ca} channel openers, strongly affecting coronary arteries but not mesenteric arteries. This specificity may provide a considerable therapeutic advantage over many other vasodilative drugs, because of inducing dilation in coronary arteries while avoiding side-effects, such as a hypotensive response, due to generalized vasodilation of peripheral resistance arteries. An additional advantage could be that K\textsubscript{Ca} channel openers may have a more desirable side effect profile compared with drugs whose targets play important roles in cardiac contractility, such as L-type calcium channel blockers or ß receptor blockers. It has to be considered that coronary arteries seem to be highly sensitive to develop endothelial dysfunction via NO impairment in presence of certain risk factors. As under conditions of NO impairment the EDHF pathway may function as a very important compensating dilative pathway to ensure adequate vasodilation and cardiac tissue perfusion (see above), the therapeutic potential of K\textsubscript{Ca} channel openers may especially lie in the activation/reinforcement of the compensatory dilative EDHF response under conditions of NO impairment.

Thus far, K\textsubscript{Ca} channel openers are not available for clinical use. For the development of specific channel modulators, the channel characteristics, and potential subtype-differences need to be explored. In this respect the large conductance K\textsubscript{Ca} channel (BK\textsubscript{Ca} channel) has already been well described. It consists of four \(\alpha\) subunits and an unknown number of \(\beta\) subunits. Coexpression of \(\alpha\) with (a) \(\beta\) subunit(s) alters biophysical properties and increases calcium sensitivity\textsuperscript{113}. Each type of \(\beta\) subunit seems to have a unique tissue distribution, and different effects on BK\textsubscript{Ca} channel pharmacology and activation gating. Therefore, the functional diversity required for the tissue-specific roles of BK\textsubscript{Ca} channels may be created in part by association with accessory \(\beta\) subunits. The \(\beta\textsubscript{1}\) subunit is especially prevalent in smooth muscle cells, whereas endothelial cells seem to lack the \(\beta\textsubscript{1}\) subunit, suggesting a substantially different channel regulation in endothelial cells compared to smooth muscle cells\textsuperscript{114}. It seems likely that subunit differences as well as differences in channel quantity also exist between arteries from different organs\textsuperscript{107,115}. This may provide the opportunity to direct therapy not only against specific tissue components, e.g. endothelial versus smooth muscle cells, but also against specific organs. Furthermore, in cardiovascular disease specific patterns of channel up-/down-regulation have been described\textsuperscript{52,107,116}. The use of gene targeting may considerably help to further define the role of specific signaling molecules and subunits in different tissues, not only of the K\textsubscript{Ca} channel but also of other types of K\textsuperscript{+} channels involved in vasoregulation. In this respect, \(\beta\textsubscript{1}\) subunit knock out mice have been generated, and the important role of the \(\beta\textsubscript{1}\) subunit in regulating calcium sensitivity of the BK\textsubscript{Ca} channel was revealed\textsuperscript{117,118}. Importantly, the targeted deletion of the gene encoding for the K\textsuperscript{+} channel subunit Kir6.1 in mice revealed a phenotype with hypercontractility of coronary arteries resembling that of Prinzmetal angina in humans\textsuperscript{119} indicating the critical role of the Kir6.1 subunit for the regulation of vascular tonus. Similarly,
deletion of the K⁺ channel subunit SUR2 in mice led to vasospastic episodes of coronary arteries.

A variety of different structural types of small-molecule compounds have been identified that possess BKCa channel agonist activity. For example, the α subunit selective benzimidazolone analog NS1619 (used in Chapter 5) stimulates channel activity by increasing the open state probability, and shifts voltage-sensitivity of the channels to more negative potentials. However, further research needs to be done to find analogs with improved selectivity as this compound seems to also interfere with other K⁺ and/or Ca²⁺ channels.

Another interesting compound is the BKCa channel opener dehydrosoyasaponin 1 (DHS-1) which preferentially targets BKCa channels on vascular smooth muscle cells, i.e. BKCa channels comprising both the α and the β₁ subunit. Interestingly, this substance is an active ingredient in Desmodium ascendenis, a medicinal herb used in Ghana as a bronchial smooth muscle dilator for the treatment of asthma.

It becomes obvious that the further evaluation of (tissue specific) KCa channel characteristics combined with the development of selective compounds with agonist activity may be of significant importance to further establish this very promising and advantageous form of vasodilator therapy.

Variability of endothelial dilator function among healthy individuals. Does it predispose some individuals for development of renal damage?

In the first part of the general discussion we have discussed disease-related alterations in endothelial function. In this respect, the finding that the severity of organ damage, i.e. infarct size relates to the development of endothelial dilator dysfunction, i.e. the severity of EDHF impairment (Chapter 3), may indicate that organ disease (via associated pathophysiological alterations) can affect endothelial function in "distant" artery types, i.e. in arteries not located in the diseased organ itself. On the other hand, bearing in mind the important role of (intact) vasomotor regulation and functioning endothelium in small intra-organ arteries for the functional integrity of the supplied organ, it may be as interesting to question, whether (individual) endothelial dilator (dys)function (before the onset of any organ damage) can predict the development of organ damage in response to certain risk factors. To treat this question was the second important aim of this thesis.

Interesting results on this topic have been obtained for the heart, describing a prognostic value of coronary endothelial vasodilator (dys)function for long-term atherosclerotic disease progression, and cardiovascular events. However, in these studies the measurement of coronary endothelial vasodilator function in individual patients may to a large extent be a reflection of the individual risk factor profile determined, amongst others, by environmental differences. Therefore, to avoid these differences in (environmental) risk factor profiles, the study of experimental animals models, under controlled conditions, may be more suitable to treat this question. In this respect, we found that in a group of normal healthy Wistar rats the endothelial dilator function (i.e. endothelium-dependent dilation to acetylcholine) was not uniformly pronounced, but was highly variable among individuals (Chapter 6). On the other hand, the susceptibility to develop organ disease varies among individual rats, despite comparable environmental conditions. Hence, a genetic component in this susceptibility has been proposed involving differences in (the expression levels of) endothelium-derived NO. We questioned therefore, whether in healthy individual rats there are
specific endothelial characteristics already present before the onset of organ disease which are associated with (and can predict) the susceptibility to develop organ damage in this individual in response to disease induction. The experimental setting for answering this is normally hampered by the difficulty that organ arteries of the organ in which the disease development has to be monitored cannot be taken out for (in vitro) investigation without damaging the organ/individual itself, as may be the case for coronary arteries and cardiac damage. Therefore, in Chapter 6, we used the 5/6 nephrectomy (5/6 Nx) model of progressive kidney disease, and we established an experimental procedure to overcome this problem. We used the kidney which is removed from the healthy rat to investigate endothelial dilator function and underlying endothelium-derived mediators in small renal (interlobar) arteries, and we followed up the development of renal damage (of the remaining kidney) in the same animal. Importantly, we found that the renal endothelial dilator function was inversely related to the severity of renal damage induced by 5/6 Nx (Chapter 6). The prognostic value was determined by the individual contribution of NO and PG, with higher individual levels of dilative NO and PGs being related to less severe renal damage after 5/6 Nx. This means that within a group of normal healthy rats, individuals with high risk for renal impairment could be detected by determination of renal dilative function. That an intact endothelial dilator function may exert a protective effect for the development of renal damage has already been proposed. However, thus far this seems to be largely based on findings on a beneficial effect of vasodilator therapy (e.g. with calcium antagonists, and ACE inhibitors) which may improve/restore endothelial dilator (dys)function one hand, and which exerts protective functions on renal disease progression on the other hand, also independent of its effect on blood pressure reduction. Furthermore, dietary supplementation with L-arginine (a precursor of NO) has beneficial effects on renal disease progression as well. In this respect, the findings reported in Chapter 6, are the first describing a prognostic impact of renal (NO and PG mediated) endothelial dilative function obtained in healthy rats for the individual susceptibility to develop renal damage. It has to be considered in this respect that the endothelium (and its mediators) exert more functions than mediating vasodilation, i.e. with anti-aggregatory, anti-proliferative, anti-adhesive, anti-oxidative, anti-apoptotic, and thereby anti-inflammatory effects. Hence, it seems to be difficult to define whether the endothelial dilative function and the dilative effect of NO and PGs of small renal arteries per se exerted a direct protective effect on the development of renal damage in our study, or whether this may rather be a reflection of other protective properties. In the latter case, the determination as mediators of endothelium-dependent dilation could rather be a marker for the presence/quantity of this (protective) compound, perhaps also in other compartments of the kidney. Nevertheless, independent of whether the measured renal artery dilation was protective per se or rather a marker for other protective mechanisms, this finding may provide important new directions as it provides the possibility to screen normal healthy individuals for high/low susceptibility for renal impairment. However, the prognostic value in our study was obtained for renal disease progression after 5/6 Nx, a form of disease induction with a high hemodynamic component, and it was obtained in rats. Therefore, it may be of significant importance to validate this finding also for other forms of renal disease, and especially in the human situation.
Future directions for individual risk assessment and strategies to prevent renal damage

If indeed the findings from Chapter 6 can be confirmed also for the clinical setting this may open important directions towards individual risk assessment for development of renal disease. For example, in the setting of renal transplantation, individual donor kidneys could undergo risk assessment, and may thereby be evaluated for their susceptibility to develop organ impairment after transplantation. Furthermore, an advanced risk management could implement the individual risk profile in the therapeutic planning, and could thereby contribute to limit the global unspecific use of (side-effect intensive) drugs. This may be even more clinically relevant when the status of renal endothelial dilator function could be indirectly determined by measurement of other (more easily accessible) artery types, such as the brachial artery. In this respect, individual flow-mediated endothelium-dependent dilation of the brachial artery in patients has been described to be a good non-invasive indicator for the presence of coronary artery disease \(^1\), but data on a potential relation between brachial and renal endothelial function have not been presented so far. Furthermore, this non-invasive tool to assess endothelial function has been demonstrated to have poor reproducibility mainly due to physiological factors, and may seriously limit the use as a screening tool for patients \(^1\). Alternatively, it may be worth to further define, whether (apart from the functional determination of NO and PG) also the molecular-biological approach can be used to predict the susceptibility to develop renal impairment, for example by determination of individual COX and/or NOS enzyme levels in different tissues.

Based on the findings from Chapter 6, it may now be interesting to find strategies to (actively) modulate the individual balance of mediators in healthy individuals, thereby potentially preventing development of organ disease by early intervention. While considering potential strategies to actively modulate endothelium-derived mediators in vivo, we were looking for dietary or drug treatment strategies which are known to affect endothelial dilator function, and which could thereby be good candidates for inducing alterations in endothelial mediator balance. In this respect ACE inhibitor therapy, especially in combination with low sodium diet, has consistently been shown to (favourably) influence endothelial dilator function in many forms of cardiovascular and renal disease \(^1\). However, the effect on endothelial function in normal healthy individuals is less well explored. Therefore, in Chapter 7 and 8, we investigated the effect of chronic ACE inhibitor therapy and sodium restriction on endothelium-derived mediators in normal healthy rats. We found that sodium restriction as well as ACE inhibition therapy (under control diet) altered the balance between NO, PG, and EDHF, while leaving the total endothelium-dependent dilation intact. Interestingly, this shift in endothelial mediator balance was differentially pronounced depending on the vascular bed studied. Hence, sodium restriction as well as ACE inhibition seem to effectively modulate endothelial mediator balance. These tools can now be used to evaluate treatment strategies of early intervention to prevent renal disease development by modulation of endothelial mediator balance.