Chapter 1

General introduction and aims of the thesis
Introduction

In recent decades, cardiovascular medicine has witnessed a tremendous progress in understanding the mechanisms governing the regulation of vascular tone. Moreover, by discovering the crucial role of the endothelium in vasomotor control\(^1\), the concept of endothelial dysfunction has been defined and the involvement of vasomotor mechanisms in the pathophysiology of cardiovascular disease and end-organ damage has been proposed\(^2-4\). Conditions characterized by chronic end-organ failure, such as cardiac or renal failure, are no more regarded as diseases of isolated organs, but rather as syndromes associated with multiple vascular changes. However, the role of peripheral and intraorgan vascular dysfunction in the initiation and progression of end-organ damage is far from being understood. Endothelial dysfunction has been shown to occur early in the course of cardiovascular disease and proposed to predict cardiovascular outcome\(^5-7\). This suggests that vascular dysfunction might be crucially involved in the development of end-organ damage. This thesis aims to investigate the role of intraorgan and peripheral vasoreactivity as a determinant of renal end-organ damage potentially representing novel renoprotective therapeutic strategy.

Vascular tone regulation

Small arteries (diameter \(< 500 \, \mu m\)) crucially regulate organ blood supply and are responsible for a major portion of the vascular resistance\(^8,9\). Vascular tone of small arteries is controlled by nervous, hormonal and local mechanisms. Reactivity of a given vascular bed is the result of interplay between vascular smooth muscle cells (VSMC) and locally produced endothelium-derived mediators. Pressurized vessels exhibit constrictive response against the intraluminal pressure, which determines the level of basal vascular tone. This reaction, termed myogenic response\(^10\), is an intrinsic property of VSMC. VSMC sense mechanical stretch, which leads to the depolarization of the cell membrane, opening of voltage-sensitive Ca\(^{2+}\) channels, subsequent Ca\(^{2+}\) influx, and activation of the contractile apparatus. On top of the basal vascular tone, determined by myogenic reactivity, vascular diameter is controlled by additional local constrictive and dilatory mechanisms. Endothelium, an inner lining of the vessel is of major importance in the regulation of vascular tone. By releasing several vasoconstrictive and dilative substances (see Chapter 2 of this thesis for details), it modulates the tone of the underlying VSMC. Endothelium-derived relaxing factors include cyclooxygenase (COX)-derived vasodilatory prostaglandins, nitric oxide synthase (NOS)-derived nitric oxide (NO), and the yet unidentified endothelium-derived hyperpolarizing factor (EDHF). Endothelial cells however also play an important role in multiple other processes, such as hemostasis, inflammation, permeability, and angiogenesis\(^11\). Improper functioning of endothelial regulation is reflected by altered release of endothelial vasodilative mediators and may be assessed by the vasodilatory response of the vessel to agonists, such as acetylcholine.
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(ACh). Impaired endothelium-mediated ACh-induced vasodilation is believed to be a marker of endothelial dysfunction, a condition associated with various aspects of cardiovascular disease. Myogenic response and endothelium-mediated reactivity represent the principal local mechanisms controlling the tone of small arteries and as such are the major focus of investigation in the present thesis.

Myogenic response and endothelial function in small renal and mesenteric arteries

Myogenic reactivity sets the basal tone of vessels and thus co-determines the basal level of resistance in the vascular tree. It is pronounced in small arteries known to serve as resistance vessels, whereas mostly absent in large conduit vessels. Small mesenteric artery, employed in our experiments, represents the prototype of a peripheral resistance artery. Increased peripheral resistance is a hallmark of several cardiovascular diseases, such as hypertension, cardiac or renal failure. Several authors have proposed that excessive myogenic reactivity might be responsible for elevated peripheral resistance in spontaneous hypertension or heart failure, whereas not much is known about chronic renal failure. However, in other organs such as the kidney and the brain, myogenic response may serve an additional function. In the kidney, myogenic reactivity of renal preglomerular arteries is responsible for renal autoregulation, a mechanism that keeps renal hemodynamics optimal under changes in systemic blood pressure. In particular, the glomerulus is protected from an increase in intraglomerular pressure and the induction of hyperfiltration. Hyperfiltration represents one of the key events in the development of proteinuria and renal end-organ damage. Indeed, several lines of evidence suggest that myogenic reactivity of renal vessels may be impaired in chronic renal failure. However, mechanisms underlying heterogeneous changes of myogenic tone between systemic and renal vasculature in chronic renal disease are not yet understood. Also, endothelial regulation may differ between intrarenal and extrarenal vascular beds. The contribution of endothelial mediators to endothelium-mediated vasodilation seems to be crucially dependent on location and the artery investigated. For instance, whereas in the large conduit arteries NO is the major endothelial vasodilator, in smaller vessels EDHF prevails. Therefore, the physiological characteristics of vasomotor regulation in a given vascular bed might be important to define their role in the pathogenesis of cardiovascular and/or renal damage. Although heterogeneity in endothelial function between renal and systemic vessels may be anticipated, it has been proposed that the kidney can be regarded as an organ reflecting generalized vascular changes, such as in case of urinary leakage of small amounts of protein (microalbuminuria), as explained below.

Chronic kidney disease- a vasculopathic state

Chronic kidney disease (CKD) refers to a condition characterized either by a decline in glomerular filtration rate (GFR of less than 60 ml/min/1.73 m²) or the presence of any other marker of renal damage, such as by histology confirmed renal injury or abnormal protein excretion. The most evident outcome of CKD is chronic renal failure requiring treatment
by transplantation and/or dialysis. However, many more individuals suffer from early stages of CKD with only mildly decreased or even normal to increased GFR. Early and late stages of CKD are currently no longer regarded as a disease of an isolated organ, but rather a vasculopathic state with generalized vascular changes in multiple vascular beds. CKD is associated with increased prevalence of cardiovascular disease and in fact, patients with CKD have even higher chance to experience a cardiovascular event than to progress to renal failure. Microalbuminuria predicts the rate of renal function decline as well as an increase in cardiovascular morbidity and mortality in several populations, giving rise to the hypothesis that excessive protein leakage in kidney is a reflection of generalized vascular or endothelial function. Moreover, it suggests that vascular changes occur in early stages of the disease and might actively participate in the development of renal and cardiovascular end-organ damage. However, the mechanisms underlying the relation between renal and systemic vascular function and their role in CKD development remain incompletely characterized.

**Figure 1.** Endothelial function of healthy rat predicts development of renal damage after 5/6 nephrectomy. Endothelium-dependent vasodilation to acetylcholine (A) and the contribution of endothelium vasodilatory mechanisms prostaglandins (B), NO (C) and EDHF (D) measured in small renal arteries of healthy rats correlate with the severity of proteinuria after subsequent 5/6 nephrectomy. Adapted from.
Predictive value of endothelial function in renal end-organ damage

The early occurrence of vascular dysfunction in the course of progressive renal disease leads to the question whether vascular function might condition the susceptibility of a healthy individual to renal damage. The severity of CKD varies considerably among patients with similar systemic risk factor profiles, such as hypertension and diabetes, and seems to be also dependent on intrinsic, probably genetically conditioned factors. Likewise, in experimental animal models of CKD, the individuals of outbred rat strains develop renal damage and renal function loss of highly variable severity after a relatively uniform injury, such as subtotal nephrectomy. In our laboratory, the hypothesis that variability in renal endothelial function among healthy animals might be responsible for the observed differences in susceptibility to end-organ damage was recently tested. To this end, endothelium-dependent vasodilation of small intrarenal arteries in vitro was measured in healthy rats, including the contribution of three principal endothelial dilatory mediators, e.g., NO, EDHF, and prostaglandins. Indeed, vascular function was remarkably variable among the individuals. Following these measurements, renal injury was induced by 5/6 nephrectomy. The endothelium-dependent vasodilation of small renal arteries predicted the subsequent development of end-organ damage, measured as proteinuria and decline in GFR. Thus, rats with more pronounced total endothelial relaxation, NO-mediated or prostaglandin-mediated vasodilation, were protected against the end-organ damage, whereas prevalence of EDHF was associated with worse renal outcome\textsuperscript{28} (Figure 1). These data suggest that variability in endothelial function among healthy individuals accounts for the differences in susceptibility to renal damage induced by a reduction in nephron number. However, it remains unknown, whether this prognostic value is specific for this particular type of renal injury or it might be extended to other forms of renal disease.

Aims of the thesis

The aim of this thesis was to investigate the role of vasomotor mechanisms in the development and progression of CKD and related systemic cardiovascular complications, providing novel potential therapeutic targets. More specifically, two principal mechanisms of vascular regulation in small arteries, namely myogenic and endothelium-mediated responses were tested in several experimental as well as spontaneous models of chronic renal disease. The following main research questions were addressed:

1. Does vascular function measured in the healthy individual (rat) predict the susceptibility of an individual to a renal insult?
2. If so, is this predictive value of vascular function dependent on the type of insult inflicted to the kidney?
3. Is renal vascular reactivity in CKD related to vasomotor function in peripheral vascular beds?
4. Does vascular function represent an early renal risk marker and a potential target for renoprotective preventive therapy?
Given the above, the following problems were studied in specific chapters of this thesis: **Chapter 2** summarizes the relation between microalbuminuria and endothelial function, providing a clinical basis for the experimental work in this thesis and suggesting potential preventive therapeutic strategies. **Chapters 3 and 4** investigate the hypothesis, that interindividual heterogeneity in renal endothelial function determines the susceptibility to experimentally-induced renal damage of various etiologies, namely myocardial infarction-induced and nephrotoxic renal damage. **Chapter 5** provides evidence of impaired renal vasomotor function in a model of spontaneous renal disease prior to the development of end-organ damage and explores the related vascular alterations in systemic vessels. **Chapter 6** addresses the role of peripheral myogenic and endothelial responses in a hypertensive CKD model. **Chapter 7** summarizes the current knowledge on renal and systemic endothelial changes in various stages of CKD, and provides experimental evidence for the predictive value of endothelial function with respect to the antiproteinuric therapeutic response by ACE inhibitors.
References

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