Small artery tone under control of the endothelium
Gschwend, Simone Katharina

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Chapter 1

Introduction
Vascular tone under control

Similar to a communication network, the cardiovascular system is a convective system that pervades the cells, provides them with vital substances and carries of their metabolic end products; in addition it transports the corpuscular elements of the blood, the respiratory gases, water and electrolytes, hormones and heat. Its efficient and complex design permits it to use a very limited volume of circulating fluid to control the chemical composition of the internal environment. An important prerequisite to maintain the constancy of the internal environment, i.e. homeostasis, is an adequate blood flow through the organ capillaries.

Blood that is ejected from the heart passes consecutively through different types of vessels which classification is based on physical dimension, morphological characteristics and specific function, as depicted in Figure 1. Apart from the principal function to conduct blood large elastic arteries (aorta + major branches, pulmonary artery, etc.), due to their distensible walls, allow to expand and receive the stroke volume during ventricular ejection, and to recoil during diastole. This converts the intermittent ejection of blood by the heart into a continuous flow through the more distal vessels.

Small arteries and arterioles

In contrast to large elastic and conduit arteries, small arteries (with a diameter of 100 µm – 500 µm) and arterioles (with a diameter < 100 µm) are characterized by much thicker walls in proportion to lumen size with more smooth muscle and less elastic material. These muscular arteries can adapt their diameter actively by constriction and dilation of vascular smooth muscle cells (VSMC), thereby actively regulating/adapting blood flow to individual demands of the organ that they supply. Even a small change in internal diameter causes a large change in vascular resistance to blood flow, owing to the fourth-power term in Poiseuille's law. Consequently, small changes in diameter strongly affect blood flow to the supplied organ with arterial constriction leading to decreased blood flow and arterial dilation leading to increased blood flow. It is this function that makes small arteries/arterioles so important for the maintenance of organ function and may explain their important role in the development of organ disease. Note that blood flow to many organs can vary over a 10-fold or larger range and this range varies a lot between different organs. For example, in the myocardium the relation of resting blood flow to maximal blood flow has been described to be approximately 1:5; in the gastrointestinal tract 1:8; and even 1:24 in the skeletal muscle.

Apart from the important function as regulators of local tissue blood flow small arteries and arterioles, due to their large resistance to blood flow, also importantly contribute to determine total peripheral resistance (Figure 1), which, in concert with cardiac output, determines arterial blood pressure (mean arterial blood pressure [mmHg] = cardiac output [ml min⁻¹] x total peripheral resistance [mmHg min ml⁻¹]). Thus, control of diameter in these so called resistance arteries exerts both local effects (control of nutritive supply and organ fluid balance), and central effects (homeostasis of blood pressure and plasma volume).

Diameter control in small arteries and arterioles: an overview

The diameter of small arteries is controlled by several interacting mechanisms. First, the vascular smooth muscle cells inherently and actively resist being stretched (as they are continuously pressurized). This constrictive response against intraluminal pressure is called myogenic or basal tone (and will be described later more in detail).

* More background information on this topic is given in Chapter 10
Large elastic arteries
- aorta + major branches
- high elastin content ensures distensible walls
- high collagen content prevents overdistension

Conduit arteries
- low resistance conduits
- thick walls prevent collapse

Small resistance vessels
- small arteries and arterioles
- due to their narrow lumen and limited number they provide main resistance to blood flow and are (co-)determinants of arterial blood pressure
- due to their thick media with large smooth muscle content they are able to turn local blood flow up or down to match local organ needs

Exchange vessels
- capillaries
- single layer of endothelial cells: transfer of metabolites

Capacitance vessels
- venules and veins

**Figure 1** Schematic drawing of blood pressure drop along the vascular tree. Effect of diameter changes in resistance vessels (adapted from Cardiovascular Physiology, McGraw-Hill ²)

From this basal tone external influences exert their dilating or constricting effects. These influences can be separated into neural, hormonal and local influences. Neural influences mainly derive from perivascular sympathetic nerves with tonic activity releasing norepinephrine, and exerting the so called neurogenic tone. Influences of circulating hormones may be of minor consequences in comparison to neural or local influences in healthy individuals under normal conditions, whereas their importance may increase under conditions of vigorous exercise, compromised body fluid balance, and in pathophysiological states such as heart failure, kidney failure and hypertension. Important circulating vasoactive hormones in this respect are (nor)-epinephrine, released from adrenal glands during activation of the sympathetic nervous system (inducing \( \alpha \)-receptor mediated constriction or \( \beta \)-receptor mediated dilation), atrial natriuretic peptide (ANP), binding to ANP receptors which are coupled to guanylyl cyclase inducing cGMP mediated dilation, as well as vasopressin (antidiuretic hormone, ADH), and angiotensin II, both very potent vasoconstrictors. Apart from circulating hormones also locally derived substances importantly contribute to set
arterial tone. They are mainly produced by the endothelium – released continuously and after stimulation – and exert constrictive (endothelin-1, thromboxane A₂, superoxides, etc.) and dilative (prostacyclin, nitric oxide, EDHF) actions. The small arteries that control blood flow through a given organ lie within the organ tissue itself. Thus, small arteries and the smooth muscle in their walls are exposed to the local chemical composition of the interstitial fluid of the organ they serve. The interstitial concentrations of many substances reflect the balance between the metabolic activity of the tissue and its blood supply. In this respect, decreased O₂ and ATP levels as well as increased adenosine, CO₂, H⁺ and K⁺ levels at high metabolic rate of the tissue, i.e. under exercise, are sensed by smooth muscle cells leading to potent arteriolar dilation.

Different vascular beds: coronary, mesenteric and renal arteries
Arteries from different vascular beds differ considerably in their sensitivity for neural, hormonal and local influences with a spectrum ranging from almost total dominance by local metabolic mechanisms to almost total dominance by sympathetic nerves⁷. For example, in small coronary arteries in vivo the normal resting arteriolar tone is high and the normal blood flow is not greatly in excess of that required to meet the normal metabolic demands of the heart tissue (Figure 2). Increasing the tissue’s metabolic rate and production of metabolically related vasodilator substances can cause a large increase in flow by removing the normally high arteriolar tone. In contrast, changes in sympathetic activity have only minor effects on coronary arteriolar tone. In mesenteric and renal arteries, however, the normal resting arteriolar tone is low and blood flow is high and well in excess of the minimum required for tissue metabolism. An increase in sympathetic activity causes a large reduction in blood flow in these organs, whereas a decrease in sympathetic activity significantly increases the blood flow. However, increasing the metabolic rate of the tissue has very little effect on blood flow. Data on the investigation of actual in vivo blood pressures in small arteries of different vascular beds are given in Chapter 10.

This regional heterogeneity of vasoregulation in different vascular beds may be a reflection of their specific functions. The main regulatory function of small coronary arteries may be to tightly adapt the blood supply to the actual (metabolic) demand of the heart, i.e. myocardial perfusion closely follows myocardial O₂ requirements. The situation may be different in small mesenteric arteries. Apart from their role in the organ's blood supply, this vascular bed furthermore acts as a functional reservoir of blood for the entire organism (which can be mobilized for example after volume loss and resulting sympathetic stimulation), and may also largely contribute to set total peripheral vascular resistance. This becomes obvious when considering that the splanchnic vascular bed receives 23% of total cardiac output (for comparison, the coronary circulation receives 4%). In contrast to coronary and mesenteric arteries, the main function of the renal blood flow is to yield a glomerular filtration rate which is sufficient for the excretory and volume-regulatory function of the kidney. This is mainly accomplished by the autoregulation of renal afferent and efferent arterioles aiming to keep the pressure in the glomerular capillaries constant (additional regulatory mechanisms are reviewed elsewhere⁸). The renal circulation receives approximately 20% of total cardiac output, and seems to be largely influenced by sympathetic activation, e.g. during hypotension and exercise.
The vascular smooth muscle $^{1,2,9}$

The degree of contraction of vascular smooth muscle cells controls the diameter of small arteries, and thereby organ perfusion. Contraction is triggered by a rise in Ca$^{2+}$ ion concentration in the cytoplasm leading to phosphorylation of myosin filaments. Contraction of vascular smooth muscle cells, in contrast to cardiac or skeletal smooth muscle cells, is characterized by a large degree of shortening, at slow velocity, sustained in nature, and low at energy cost (1/300 of the energy expenditure of striated muscle). The sarcoplasmic reticulum, a releasable store of Ca$^{2+}$ ions, is poorly developed in small resistance arteries as compared to large conductance arteries. Therefore, small arteries very much depend on extracellular Ca$^{2+}$ and the Ca$^{2+}$ influx through voltage-sensitive Ca$^{2+}$ channels (VSCCs), and are therefore very sensitive to VSCC blockers such as nifedipine. The smooth muscle membrane potential usually is in a relatively depolarized state, around -60 mV to -50 mV, and the membrane is well endowed with VSCCs whose open state increases steeply with depolarization. Therefore, the degree of contraction of most resistance arteries very much depends on the smooth muscle membrane potential with depolarization causing constriction and hyperpolarization causing dilation. As small arteries are the major regulators of blood flow to the end-organs, organ perfusion is exquisitely sensitive to the membrane potential of the smooth muscle of the supplying arteries. At -60 to -50 mV the VSCCs have a low but finite open probability which allows a small but finite influx of extracellular Ca$^{2+}$ into the cell to generate basal tone/constriction. As seen in Figure 3, increasing intraluminal pressure further depolarizes the membrane $^{10,11}$, may be due to activation of stretch-activated cation channels, leading to opening of VSCCs, Ca$^{2+}$ influx and the so called myogenic...
Chapter 1

**constriction**. This basic mechanism of the myogenic response may certainly be further modulated by additional signal transduction pathways such as activation of protein kinase C and Rho kinase, which may increase the Ca\(^{2+}\) sensitivity of the contractile apparatus. Furthermore, constriction may be limited by negative feedback mechanisms, as the K\(_{Ca}\) channel-mediated hyperpolarization via Ca\(^{2+}\) sparks. A decrease in intraluminal pressure induces the opposite response leading to dilation of the artery. The myogenic mechanism, first described in 1902 by Bayliss, serves to control blood flow to organs after acute pressure changes, and may prevent organ damage of critical organs (heart, brain, kidney) after acute increases in blood pressure. A detailed review on the current status of research on the myogenic mechanism is given by Davis et al.

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*Figure 3* Myogenic activation of vascular smooth muscle cells. Pressure induces membrane depolarization leading to opening of voltage-sensitive calcium channels (VSCCs) and cytosolic Ca\(^{2+}\) increase. Pressure-induced activation of phospholipase C and other second messenger systems (inositol triphosphate (IP3); diacylglycerol (DAG); protein kinase C, Rho kinase) leads to increased Ca\(^{2+}\) sensitivity of the contractile apparatus. Increased Ca\(^{2+}\) concentration as well as Ca\(^{2+}\) sensitivity induce smooth muscle contraction. Contraction is counteracted by "Ca\(^{2+}\) sparks" leading to K\(_{Ca}\) channel-mediated hyperpolarization and dilation. SR, sarcoplasmic reticulum.

The basic mechanism of contraction shown in *Figure 3* may also count, in a modified form, for agonist-/receptor-induced constriction to substances such as angiotensin II, endothelin-1, serotonin and norepinephrine, although the different signaling pathways together leading to vasoconstriction may be more or less pronounced depending on the type of agonist, but also

* More background information on this topic is given in Chapter 10
depending on the artery type and size [22-25]. Importantly, in addition to membrane depolarization-induced Ca\(^{2+}\) influx via VSCCs (electro-mechanical coupling) agonists may induce Ca\(^{2+}\) increase and contraction mainly by agonist-specific receptor mediated activation of second messengers, and receptor-operated cation channels (ROC) (pharmaco-mechanical coupling). Nevertheless, due to the considerable overlap of signaling mechanisms leading to pressure-induced and agonist-induced constriction it has been hypothesized that both mechanisms may influence/amplify each other in a way that the presence of an agonist may enhance pressure-induced constriction, and vice versa. Indeed, it has been found that subthreshold concentrations of angiotensin II as well as norepinephrine significantly enhance the myogenic response to pressure increase [21,26,27], and furthermore, an increase in intraluminal pressure enhances the constrictive response to agents such as norepinephrine [28] and endothelin-1 [29].

The endothelium

Endothelial cells cover the entire inner surface of the cardiovascular system, thereby representing a critical strategic interface between blood and tissue. The location and the enormous surface area enable the endothelium to interact very effectively with blood components but also with adjacent vascular smooth muscle cells. Hereby, the endothelium is able to sense changes in hemodynamic forces or blood-borne signals by membrane receptor mechanisms, and responds by synthesis and release of a variety of vasoactive mediators. The endothelium plays a very important role in the regulation of blood hemostasis, inflammation, permeability, and angiogenesis, which has been reviewed elsewhere [30]. Furthermore, the endothelium is an important regulator of vascular tone by releasing a variety of contractile and relaxing factors, which exert their effects on underlying smooth muscle cells [31-33].

Endothelium-derived contracting and relaxing factors

As seen in Figure 4, endothelium-derived contracting factors [31] include the peptide endothelin-1 (ET-1) [34], vasoconstrictor prostanoids such as thromboxane A\(_2\), and prostaglandin H\(_2\), as well as superoxide anions and angiotensin II, the latter of which is converted from angiotensin I on the luminal surface of endothelial cells by angiotensin-converting enzyme (ACE). Endothelium-derived relaxing factors [31] (Figure 4) include vasodilative prostaglandins* (mainly prostaglandin I\(_2\)), nitric oxide* (NO), and the so called endothelium-derived hyperpolarizing factor* (EDHF). Prostaglandin I\(_2\) (PGI\(_2\), prostacyclin) is formed from arachidonic acid following the activation, in turn, of phospholipase A\(_2\), cyclooxygenase (COX), and prostacyclin synthase. Prostaglandin I\(_2\) production can be effectively inhibited by indomethacin, a potent inhibitor of COX. Prostaglandin I\(_2\) mediates dilation mainly by the cAMP pathway. The best known endothelium-derived relaxing factor is NO. NO is synthesized following the conversion of endothelial L-arginine into L-citrulline by (different isoforms of) the enzyme NO synthase (NOS). NO then diffuses to the underlying smooth muscle cells, and mediates dilation mainly by the cGMP pathway. The production of NO can be effectively inhibited by L-arginine analogues such as N\(_\text{G}\) monomethyl-L-arginine (L-NMMA [35]) which compete with the natural precursor L-arginine at the catalytic site of the enzyme.

* More background information on this topic is given in Chapter 10
Figure 4 Principal mechanism of constriction and dilation by endothelium-derived factors; ACE, angiotensin-converting enzyme; soluble AC, soluble adenylate cyclase; cAMP, cyclic adenosine-3',5'-monophosphate; AT₁, angiotensin II type 1 receptor; ATP, adenosine triphosphate; COX, cyclooxygenase; DAG, diacylglycerol; ECE, endothelin-converting enzyme; EDHF, endothelium-derived hyperpolarizing factor; ETₐ, endothelin type A receptor; ET₁, endothelin-1; FAD, flavin adenine dinucleotide; soluble GC, soluble guanylate cyclase; cGMP, cyclic guanosine-3',5'-monophosphate; GTP, guanosine triphosphate; IP₃, inositol 1,4,5-trisphosphate; m-receptor, muscarinic receptor; NO, nitric oxide; NOS, NO synthase; PGI₂, prostacyclin; PKC, protein kinase C; PLC, phospholipase C; TH₄, tetrahydrobiopterin; TXA₂, thromboxane A₂ receptor.

Functional assessment of endothelial (dys-)function: the ACh test
The release of endothelium-derived vasoactive factors is triggered by a host of mediators, including bradykinin, acetylcholine (ACH)*, and by hemodynamic forces such as shear stress. Under physiological conditions, a precise and balanced release of relaxing and contracting factors ensures adequate organ perfusion. Alterations in this balance, for example a decreased production of dilative mediators or an increased production of constrictive mediators, lead to impaired vasodilation with considerable effects on organ perfusion and vascular resistance, and hence organ function and blood pressure. Such an imbalance between relaxing and contracting factors, but also between anti- and pro-coagulant, and growth-inhibiting and

* More background information on this topic is given in Chapter 10
growth-promoting mediators of the endothelium, is commonly referred to as endothelial dysfunction \(^{30}\). To functionally investigate the ability of the endothelium to produce (and release) vasodilative mediators the following experimental approach is generally used. Arteries, in vivo or in vitro, after being precontracted to a steady level, are exposed to increasing concentrations of an endothelium-dependent dilator, most often a muscarinic agonist (such as ACh) or bradykinin, and the dilative response of the arteries is determined. An intact production and release of dilative mediators may be reflected by an intensive dilative response to ACh, and may be characteristic for a functioning and healthy endothelium. In contrast, the functional evidence of a decrease in the maximum and/or a reduced sensitivity of the dilative response to ACh - under conditions of a preserved response to endothelium-independent dilators - is considered to reflect endothelial dysfunction. A critical review on the ACh test and its interpretation is given by Angus et al. \(^{36}\).

To further characterize alterations in the endothelial mediators underlying the endothelium-dependent dilation, the dilative response to ACh can be additionally measured in presence of potent inhibitors of the production of these mediators, i.e. indomethacin to inhibit prostaglandins, and L-NMMA to inhibit NO \(^{35}\). In this respect, the more the response to ACh is attenuated in presence of the inhibitor, the more this mediator is considered to contribute to mediate the ACh dilation. However, even in case of (effective) blockade of the production of prostacyclin and NO, there is still a considerable dilative response to ACh left especially in arteries of smaller size \(^{37,38}\), the so called indomethacin- and L-NMMA-resistant relaxation. This introduced the idea of the presence of a third (or even more) endothelium-derived dilative mediator(s).

**Endothelium-derived hyperpolarizing factor (EDHF)**

Bolton et al. found, in 1984, that muscarinic receptor stimulation of endothelial cells was associated with hyperpolarization of underlying smooth muscle cells \(^{39}\), which was also confirmed by others \(^{40-42}\). Later it was shown that the indomethacin- and L-NMMA-resistant relaxation to ACh was tightly associated with this hyperpolarizing response of smooth muscle cells, and therefore the term *endothelium-derived hyperpolarizing factor (EDHF)* \(^*\) was coined \(^{43-47}\). An extensive amount of research has been performed during the last years to identify the (chemical) nature of EDHF (reviewed by \(^{48-51}\)). Thus far, there is still no single factor found which can describe the EDHF phenomenon in all different artery types and species, which is described more in detail in Chapter 10. However, it could be demonstrated very consistently, and rather independent of investigated artery types and species, that the indomethacin- and L-NMMA-resistant relaxation, and the associated hyperpolarization of smooth muscle cells, can be effectively blocked by a combination of *potassium channel blockers*, i.e. the \(K_{Ca}\) channel blockers charybdox toxin plus apamin \(^{52-55}\). Therefore, to experimentally study the contribution of EDHF to endothelium-dependent dilation the dilative response of arteries to ACh is measured in absence and presence of this toxin combination and again, the more the toxin combination inhibits the dilative response to ACh, the more EDHF is considered to contribute to mediate ACh dilation. This experimental approach is complicated, however, by two facts. First, not only EDHF but also NO may exert its effect partly via activation of (charybdox toxin-sensitive) potassium channels \(^{56-59}\). Therefore, when using charybdox toxin and apamin, not only EDHF but also the potassium channel-mediated part of NO-mediated dilation may be inhibited. Secondly, several (inhibitory) interactions between prostaglandins, NO and EDHF have been described, and importantly, NO is believed to be able to suppress EDHF \(^{60-62}\). This

\(^*\) More background information on this topic is given in Chapter 10
may imply that EDHF only becomes fully active when NO is blocked, and this has to be taken into account when performing and interpreting measurements on EDHF-mediated dilation. One experimental approach to circumvent the influence of those interactions may be to determine EDHF-mediated dilation only in presence of inhibitors of prostaglandins and NO, i.e. indomethacin and L-NMMA.

Role of vascular ion channels in mediating constriction and dilation

Ion channels on vascular smooth muscle cells\(^{1,2,9}\)

In contrast to large arteries, smooth muscle cells of small arteries and arterioles are well endowed with voltage-sensitive calcium channels (VSCCs, approximately 1000 channels per cell). The special characteristic of these channels is that their open probability is a continuous steep function of the membrane potential. At the cells typical membrane potential (approximately -50 mV) a 10 % change in membrane potential causes a three fold change in open state probability. Since there is a large Ca\(^{2+}\) gradient into the cell (Table 1), depolarization - and hence opening of VSCCs - causes a large influx of extracellular calcium leading to arterial constriction. Therefore, due to the high number of VSCCs small artery tone is strongly dependent on membrane potential with depolarization (shift to less negative potentials) causing constriction, and hyperpolarization (shift to more negative potentials) causing dilation.

Furthermore, the smooth muscle membrane contains large amounts of K\(^+\) channels (around 50000 per cell). The importance of these channels is that by controlling the membrane potential, and hence the open probability of the VSCCs, they influence vascular tone and resistance\(^63\). There are many different types of K\(^+\) channels whose open probability seems to be sensitive to several stimuli (Table 2). Opening of K\(^+\) channels leads to an outward flux of K\(^+\) ions due to the electro-chemical gradient for K\(^+\) (Table 1), and leaves behind the net negative charge, i.e. hyperpolarizes the cell membrane. As described above, hyperpolarization of the smooth muscle cell membrane decreases the open probability of VSCCs, and thereby causes a decrease in Ca\(^{2+}\) concentration leading to arterial dilation.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Intracellular [mM]</th>
<th>Extracellular [mM]</th>
<th>Nernst equilibrium potential [mV]</th>
<th>Direction of electro-chemical gradient at –50 mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>K(^+)</td>
<td>165</td>
<td>5</td>
<td>-89 (^*)</td>
<td>out of the cell</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>0.0001 (relaxed (\S))</td>
<td>1.2 (ionic)</td>
<td>+124</td>
<td>into the cell</td>
</tr>
</tbody>
</table>

\(^*\) derived from the Nernst equation, meaning that at –89 mV membrane potential the chemical outward gradient for K\(^+\) equals electrical inward gradient.

\(\S\) the intracellular calcium concentration must rise to 0.0005 – 0.0018 mM to elicit contraction\(^64\).

In this respect, calcium-dependent K\(^+\) (K\(_{Ca}\)) channels on smooth muscle cells are very important for the control of small artery tone and fine-tuning of agonist- and pressure-induced (myogenic) constriction. K\(_{Ca}\) channels are activated by intracellular calcium and by
Table 2 Potassium and calcium channels in smooth muscle and endothelial cell membranes.

<table>
<thead>
<tr>
<th>Channel</th>
<th>Short description</th>
<th>Inhibitor</th>
<th>Activator</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vascular smooth muscle</strong> (reviewed by Nelson et al. 90)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium channels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{IR}$</td>
<td>Inwardly-rectifying</td>
<td>Set resting membr. potential</td>
<td>$\text{Ba}^{2+}$, TEA, Cs$^+$</td>
</tr>
<tr>
<td>$K_V$</td>
<td>Voltage-dependent</td>
<td>Set resting membr. potential</td>
<td>4-AP</td>
</tr>
<tr>
<td>$K_{ATP}$</td>
<td>ATP-sensitive</td>
<td>Sense metabolic state of tissue</td>
<td>Glibenclamide</td>
</tr>
<tr>
<td>$K_{Ca}$</td>
<td>Calcium-dependent large-conductance (&gt;100 pS)</td>
<td>Neg. feedback brake on depolarization / constriction Set membr. potential Mediates EDHF-response (?)</td>
<td>Iberiotoxin (spec.) Charybdotoxin TEA</td>
</tr>
<tr>
<td><strong>Calcium channels</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSCC</td>
<td>L-type voltage-sensitive</td>
<td>$\text{Ca}^{2+}$ influx tightly controlled by membrane potential</td>
<td>Nifedipine Diltiazem</td>
</tr>
<tr>
<td>ROC</td>
<td>Receptor-operated</td>
<td>Agonist-induced cation influx, e.g. to AngII, PE</td>
<td></td>
</tr>
<tr>
<td><strong>Endothelium</strong> (reviewed by Nilius et al. 70)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{Ca}$</td>
<td>Calcium-dependent small-conductance (4-20 pS) intermediate-conductance (20-60 pS)</td>
<td>Set membr. potential Mediate EDHF-response (?)</td>
<td>Apamin Scyllatoxin Charybdotoxin Clotrimazole</td>
</tr>
<tr>
<td>$K_{ATP}$</td>
<td>ATP-sensitive</td>
<td>See above</td>
<td>See above</td>
</tr>
<tr>
<td>$K_{IR}$</td>
<td>Inwardly-rectifying</td>
<td>Set membr. potential Mediate $K^+$ efflux to shear forces Sensitive to small $K^+$ increases (EDHF?)</td>
<td>See above</td>
</tr>
<tr>
<td>Non-selective cation channels</td>
<td>Mechano-sensitive</td>
<td>$\text{Ca}^{2+}$/influx/$K^+$ efflux to stretch/shear forces</td>
<td>Gadolinium</td>
</tr>
<tr>
<td>ROC</td>
<td>Receptor-operated</td>
<td>Agonist-induced cation/calcium influx, e.g. to ACh</td>
<td></td>
</tr>
</tbody>
</table>

ACh, acetylcholine; Ang II, angiotensin II; 4-AP, 4-aminopyridine; ATP, adenosine triphosphate; DHS-I, dehydrosoyasaponin; PE, phenylephrine; TEA, tetraethylammonium chloride; spec., specific.
depolarization. Since open K$_{Ca}$ channels cause hyperpolarization, and are activated by (agonist/pressure-induced) depolarization, they may exert a stabilizing, negative feedback on the membrane potential, and may thereby counteract contraction (see also Figure 3). This research field has been extensively explored by the group of Nelson et al., who demonstrated the important role of Ca$^{2+}$ sparks in this response. In addition, several studies have demonstrated a quite different role of smooth muscle K$_{Ca}$ channels in the myogenic response, describing that active closure of K$_{Ca}$ channels contributes to the initiation of myogenic depolarization and constriction. This would involve substances such as the cytochrome P450-derived eicosanoid 20-HETE which may be produced in smooth muscle cells in response to agonists or stretch. On the other hand, K$_{Ca}$ channels may not only play a role in modulating constrictive responses but may also be involved to mediate the dilative EDHF response. It has been proposed that endothelial EDHF-like substances such as the cytochrome P450-derived eicosanoid 11,12 epoxyeicosatrienoic acid (11,12 EET) diffuse to the underlying smooth muscle cells causing an opening of K$_{Ca}$ channels on the smooth muscle membrane, thereby leading to dilation of the artery. Although there is evidence that this may indeed account for the EDHF response in coronary and renal arteries of several species, quite different mechanisms have been suggested to underlie the EDHF-response in other artery types such as the rat carotid and hepatic artery, in which the K$_{Ca}$ channels involved in the EDHF response seems to be located on endothelial cells (see also Chapter 9 and 10). To experimentally study the involvement of ion channels in the regulation of arterial tone several specific inhibitors and activators/openers are available which are summarized in Table 2, and which are described more in detail in Chapter 10.

**Ion channels on endothelial cells**

The production of endothelium-derived mediators prostacyclin*, NO*, and EDHF* depends on a rise in cytosolic Ca$^{2+}$ ion concentration in endothelial cells, which is brought about by influx of extracellular Ca$^{2+}$ ions, and partly by release of intracellular Ca$^{2+}$ stores. Importantly, endothelial cells do not possess (or possess a very low amount of) VSCCs. Therefore, in contrast to vascular smooth muscle cells, Ca$^{2+}$ influx may be less triggered by direct effects of membrane potential on open probability of Ca$^{2+}$ channels. The channels through which Ca$^{2+}$ enters the endothelium seem to be on one hand receptor-operated, non-selective cation channels (ROC) which are activated by the binding of agonists such as acetylcholine*, bradykinin, and histamine to their specific membrane receptors. On the other hand, mechanosensitive (stretch-activated) non-selective cation channels may increase permeability to Ca$^{2+}$, thereby increasing intracellular Ca$^{2+}$ concentration in response to mechanical stimuli such as shear forces (studied by the group of Hoyer and Köhler et al.). The amount of Ca$^{2+}$ influx through receptor-operated channels may be influenced by the membrane potential, although not through direct effects on the open probability of the channels, but because a hyperpolarized (highly negatively charged) membrane will increase the electrical gradient for Ca$^{2+}$ to enter the cell. For example, in a depolarized endothelial cell, agonists such as histamine evoke less of a rise in intracellular Ca$^{2+}$ concentration (and hence, production of endothelium-derived mediators), and vice versa.

Not only vascular smooth muscle membranes, but also endothelial cell membranes possess K$_{Ca}$ channels, which are activated by a rise in intracellular Ca$^{2+}$ and depolarization. However, the channel conformation seems to be different, i.e. K$_{Ca}$ channels on endothelial cells have

* More background information on this topic is given in Chapter 10
been shown to lack the $\beta_1$ subunit, and are therefore less $\text{Ca}^{2+}$ sensitive compared to smooth muscle $\text{KCa}$ channels. Agonists such as acetylcholine, which raise intracellular $\text{Ca}^{2+}$, will open endothelial $\text{KCa}$ channels leading to $\text{K}^+$ efflux and hyperpolarization of endothelial cells. In contrast to smooth muscle cells however, this hyperpolarization may not similarly counteract $\text{Ca}^{2+}$ influx, as the majority of endothelial $\text{Ca}^{2+}$ channels are not voltage-sensitive, but the hyperpolarization will rather increase $\text{Ca}^{2+}$ influx through receptor-operated calcium channels, because hyperpolarization enhances the electrical gradient for $\text{Ca}^{2+}$ to enter the cell. In small arteries/arterioles the hyperpolarization of endothelial cells can be transmitted to the underlying vascular smooth muscle through myoendothelial gap junctions (reviewed by Christ et al. and Beny et al.). Importantly, this phenomenon has been proposed to (at least in part) underlie EDHF-mediated dilation in specific artery types, e.g. mesenteric arteries. It is increasingly recognized that in small arteries/arterioles gap junctions may play an important role in the spread of electronic potentials as well as small signaling molecules such as $\text{Ca}^{2+}$ and inositoltrisphosphate (IP3). This spread occurs from endothelial to endothelial cell, and from smooth muscle to smooth muscle cell (homocellular gap junctions), as well as from endothelial cells to underlying smooth muscle cells, and vice versa (myoendothelial gap junctions), and may serve to coordinate the response of a group of cells to a stimulus. In line with this synchronized oscillations have been observed in groups of cultured endothelial cells that can be prevented by uncouplers of gap junctions. Furthermore, simultaneous oscillations in the electrical potentials of endothelial and smooth muscle cells have been observed. An interesting hypothesis in this respect is that specific cells may not be equally responsive to a specific stimulus (agonist or stretch, etc.), and that gap junctional coupling may transmit electrical and/or chemical signals from directly activated cells to those cells that are more insensitive to a given stimulus.

* More background information on this topic is given in Chapter 10
Small artery function in progressive organ disease: a vicious circle

A large amount of literature is available describing alterations in contractile and/or dilative function of arteries in association with human cardiovascular and renal disease (reviewed by Rubanyi et al. [30], Elliott et al. [91], Drexler et al. [92], Adamopoulos et al. [93], and Searle et al. [94]). This counts for intra-organ arteries as well as for distant artery types, i.e. artery types not located in the organ itself, for small arteries as well as for large arteries. However, in most of the cases the nature of interactions between organ disease and arterial alterations, and hence, cause-effect relations are unclear [91, 95]. In this respect, different forms of artery-organ interactions may be considered, which will be described more in detail, and which are schematically depicted in Figure 5.

Small artery dysfunction precedes and promotes organ disease

Small artery diameter is a major determinant of organ tissue blood flow, and several control mechanisms of small artery diameter ensure adequate adaptations of organ perfusion to individual demands of the organ [13]. Alterations in these control mechanisms, e.g. an overweight of constrictive influences compared to dilative influences, commonly referred to as "small artery dysfunction", may directly affect the blood supply to the organ, and can promote harmful alterations in the organ’s functional integrity. With the discovery of the endothelium as a highly active metabolic and endocrine organ, together with the establishment of the important role of the endothelial dilative mediator nitric oxide (NO) in the eighties, the attention was very much focussed on the role of endothelial cells and NO production in disease [30, 94, 96, 97]. In line with this the idea of a protective action of functioning endothelium and its dilative action on the development of organ disease has been formulated. In support of this, it has been well established that an impaired endothelium-dependent dilation, and impaired endothelial NO production in coronary arteries - associated with the presence of several atherosclerotic risk factors - precedes the development of coronary atherosclerosis, and ischemic manifestations of coronary artery disease, leading to progressive deterioration in cardiac function [98-102]. A similar mechanism has been proposed for the kidney [103], although direct evidence for a protective role of renal artery endothelium on progressive renal failure has not yet been established.

Organ disease promotes small artery dysfunction of other organ circulations

Due to the complex interplay of neurogenic, humoral and local mechanisms controlling the homeostasis of the entire organism, the specific function of one organ will always more or less directly affect the important role of the entire organism, and therefore other organs and their (intra-organ) circulations (Figure 5). In this respect, coronary artery disease (CAD) in association with renal failure has been observed, contributing to progressive deterioration in cardiac function [104-107]. However, the underlying mechanisms of the increased incidence of CAD in renal disease has not yet been clarified. Apart from renal failure also cardiac failure has been shown to be associated with alterations in several distant artery types [92, 108], such as small femoral and mesenteric arteries [109], artery types which considerably contribute to set total peripheral resistance.

Increased total peripheral resistance reinforces organ disease progression

The vasomotor function of artery types such as the femoral or the mesenteric artery are important determinants of total peripheral resistance, and functional alterations in these arteries directly or indirectly (via neurohumoral activation) influence the function of (all)
other organs and their circulations (Figure 5). For example, an increased constrictive state of these arteries enhances the pressure work of the heart which may be deleterious especially under conditions of already impaired cardiac function, and may thus contribute to reinforce cardiac disease progression\(^{110}\). Furthermore, increased total peripheral resistance, and hence blood pressure, may on the long-term be also harmful for kidney function.

As becoming obvious from the above, due to considerable interactions between organ artery function, organ function, as well as total peripheral resistance, any single alteration will co-affect many other parameters resulting in a complex interplay, progressive in nature, and in which it may be hard, or sometimes impossible, to define cause-effect relations, especially at an advanced state of this interplay. This may be especially the case in the human situation, and may emphasize the need/advantage to employ experimental animal models for specific diseases to investigate underlying mechanisms of artery-organ interactions under controlled conditions.

**Endothelium-derived mediators involved: NO versus EDHF**

Since recently, and especially in line with the progress of in vitro investigations of arteries with smaller size\(^ {38}\), it has been more and more recognized that NO may not be the sole substance responsible for endothelium-dependent dilation and its impairment. In contrast to large arteries in which endothelium-dependent dilation is indeed mainly mediated by NO, in small arteries and arterioles a large amount of the dilative response seems to be mediated by *endothelium-derived hyperpolarizing factor(s)* (EDHF)\(^ {37,38}\). Furthermore, NO has to be very much regarded with respect to its interactions with the other endothelium-derived mediators, particularly EDHF. Importantly, an impaired NO production does not necessarily lead to impaired endothelium-dependent dilation, because EDHF has been shown to adaptively increase when NO is impaired\(^ {60,61,111-114}\). Therefore, the functional definition of endothelial dysfunction as an impaired endothelium-dependent dilation, may have to be widened (especially in small arteries) with respect to the contribution of each of the three individual endothelium-derived mediators NO, EDHF, and prostacyclin, and the specific balance among them. Although not necessarily leading to an impairment of endothelium-dependent dilation, a shift in the balance between the mediators, e.g. towards less NO/more EDHF, might be a first indication of (developing) endothelial dysfunction. On the other hand, especially in small arteries, an impaired EDHF response itself could importantly contribute to an attenuated endothelium-dependent dilation. Furthermore, with decreasing vessel size not only EDHF becomes more pronounced, but also the myogenic (constrictive) responsiveness of arterial smooth muscle in response to pressure\(^ {115}\). Importantly, the EDHF as well as the myogenic response seem to rely on similar regulatory mechanisms, i.e. both exert their action by ion channel modulation leading to membrane potential alterations of smooth muscle cells, and diameter changes. It may be speculated therefore, that in small arteries, both mechanisms may influence each other, which could be important for small artery function and its role in cardiovascular and renal disease.

\(^{*}\) More background information on this topic is given in Chapter 10
This thesis

Scope
The aim of the present thesis is to investigate the role of small artery function in organ disease progression, particularly of the heart and kidney, and to explore potential treatment strategies. More specifically, endothelial function and myogenic responsiveness are studied in pressurized small arteries obtained from animal models representing specific artery-organ interactions. Special emphasis is given to establish and analyze the balance of endothelium-derived mediators in different vascular beds in normal and diseased conditions, potential interactions of endothelium-derived mediators with the myogenic response, and to develop treatment strategies to influence these endothelial mediator balances for therapeutic reasons.

Figure 5 Complex interplay between organ artery function, organ function, and total peripheral resistance. The numbers indicate the Chapters in which the according animal model of artery-organ interaction was used.

Models of small artery-organ interactions
Figure 5 presents a hypothetical scheme in which we have tried to summarize and simplify the complex interplay between organ artery function, organ function, and peripheral vascular resistance, as discussed earlier, and as we believe to often occur simultaneously in human renal and cardiovascular disease. Subsequently, we identified a number of different experimental animal models each of which more or less accounting for specific steps in our scheme. These models were employed as a general strategy to provide further evidence for specific interactions, and to characterize these in terms of small artery function. Given the recent interest and increasing evidence for the predisposition of renal (dys-)function on future cardiac events, we employed the Munich Wistar Frömter (MWF) rat model* 116-118 of spontaneous albuminuria, and investigated coronary (and mesenteric) arteries of these rats, compared to appropriate controls without albuminuria (Chapter 2).

* More background information on this topic is given in Chapter 10
Given the increased total peripheral vascular resistance in chronic heart failure (CHF) which is believed to contribute to further progression of the disease, we employed the rat coronary ligation-myocardial infarction model. In this model, rats that are free of risk factors or pre-existing organ dysfunction are acutely subjected to severe and persistent loss in cardiac function, resulting in chronic heart failure (CHF) development in absence of confounding factors, and we investigated different types of (resistance) arteries (Chapter 3+4).

To account for a potential effect of intra-organ small artery function on the development of organ impairment we used the 5/6 nephrectomy model*, and we took advantage of the fact that the kidney providing the small renal artery to be studied for arterial function is removed from the healthy animal as a part of the model to subsequently induce progressive renal damage (Chapter 6).

Focus on balances of endothelium-derived mediators, myogenic responsiveness, and heterogeneity of endothelial dilator function

It should be recognized from earlier discussions that when studying small artery function, the specific functional characteristics of small arteries have to be taken into account. In this respect, endothelial function requires definitions of endothelial dysfunction that go beyond impaired endothelium-dependent dilation and reduced NO activity. Given the increasing importance of EDHF-type dilation and myogenic responsiveness as artery size decreases, this appears to be particularly true for small artery function in the present thesis. Therefore, we extended the investigation on endothelium-derived mediators particularly with EDHF additionally to NO, taking into account the balance between these mediators in addition to absolute dilation capacity, and having in mind potential interactions with myogenic mechanisms. Furthermore, endothelial function may be rather heterogeneous among different vascular beds with respect to the relative importance of different endothelial mediators, and this could play a role in the different sensitivities of specific artery types for disease related impairment. In addition to that, endothelial function seems to be rather heterogeneous among (healthy) individuals not only with respect to absolute differences in endothelium-dependent dilation, but also the balance of different endothelial mediators for a given artery type, and such differences could play a role in the different susceptibilities of individuals to develop organ impairment. Finally, the development of therapeutic intervention towards modulation of endothelial mediator balance may be a useful tool not only for potential therapy of disease-related alterations, but also for potential protective therapy in forms of early intervention.

Specific research objectives

With reference to the above settings of small artery-organ interaction, and extended definitions of endothelial function for small arteries, we focus in the first part of this thesis on disease-related alterations in small artery function, and we explore mechanisms underlying these alterations in specific vascular beds. This resulted in the following specific research questions:

-Chapter 2: Can a setting of mild renal organ dysfunction give rise to loss in endothelial function in small coronary arteries as to explain the relative high incidence of cardiovascular complications in renal disease?

* More background information on this topic is given in Chapter 10
- Chapter 3: Is there any evidence that the extent of cardiac damage and organ failure (infarct-size) determines how and to which extent endothelial function becomes deteriorated?
- Chapter 4: Does severe and persistent loss in cardiac function per se in absence of confounding factors give rise to increased myogenic responsiveness of small resistance arteries that may explain increased peripheral vascular resistance in CHF and its progressive nature?
- Chapter 5: What is the relationship between myogenic responsiveness and the balance status of endothelium-derived mediators. Does this involve a common mechanism which can be used for interventional therapy?

In the second part of the thesis we focus on the endothelial mediator balance in healthy rats, and on its variability among individuals, as to define endothelial characteristics of small intra-organ arteries predisposing for development of organ disease, specifically for renal arteries and renal disease development. Afterwards possibilities for modulation of endothelial mediator balance in healthy rats are explored with view on potential protective therapy. This resulted in the following specific research questions:
- Chapter 6: Can the balance status of endothelium-derived mediators in small intra-renal arteries have a prognostic value for the extent to which the renal organ damage develops following a noxious intervention inducing renal function loss?
- Chapter 7: How does low dietary sodium compared to normal sodium intake affect the balance status of endothelium-derived mediators?
- Chapter 8: How does chronic ACE inhibitor therapy affect the balance status of endothelium-derived mediators, and is this differentially pronounced during low dietary sodium intake?

The contents of Chapter 2-8 are schematically depicted in Figure 6.
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