Antioxidant vasorelaxant effects of the never beta-adrenoreceptor antagonist

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CHAPTER 8

General discussion and conclusions

Summary
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In the present thesis, we investigated various aspects of the $\beta_1$-selective adrenoceptor antagonist nebivolol in different pathological and non-pathological animal models. We thereby focused on the two additional properties in which nebivolol differs from most other $\beta_1$-selective antagonists: its vasodilator activity and its potency as an antioxidant. Indeed, nebivolol does not only exert an effect on cardiac $\beta_1$-receptors; in many animal and human vascular beds it has been shown to induce vasodilatation directly or to enhance endothelium-dependent vasodilatation mediated by the neurotransmitter acetylcholine. It is generally assumed that nebivolol exerts these actions via stimulation of the nitric oxide (NO) pathway, leading to the formation of cGMP. Indeed, our experiments performed in the rat aorta (Chapter 2) were in agreement with this assumption: nebivolol induced a concentration-dependent vasorelaxation that could be blocked by mechanical removal of the endothelium and by administration of L-NNA, an inhibitor of nitric oxide synthase. Furthermore, we investigated the role of serotonergic as well as $\beta$-adrenergic pathways in nebivolol-induced vasconstriction. Interestingly, in the rat aorta, the vasorelaxant action of nebivolol appeared to be partly mediated by a $\beta_3$-adrenergic pathway. A serotonin-mediated pathway appeared not to play a role.

Experiments performed in the rat coronary vasculature, however, demonstrated that neither L-NNA, nor ODQ, an inhibitor of guanylyl cyclase, influenced nebivolol-induced vasorelaxation (Chapter 3). These findings suggest that at least in the rat coronary bed, nebivolol-induced vasodilatation is not endothelium-dependent. Furthermore, in the rat coronary bed, we could not demonstrate the involvement of serotonergic- or $\beta$-adrenergic receptor pathways. Consequently, in the coronary vasculature, the precise mechanism of nebivolol-induced vasorelaxation remains to be defined.

In order to investigate the vasorelaxant activity of nebivolol in two relevant pathological models, that is hypertension and congestive heart failure, we studied its efficacy in isolated aortic rings obtained from both spontaneous hypertensive rats (SHR) and from rabbits with experimental heart failure, respectively. In both models, we were able to demonstrate that endothelial function was significantly impaired, as reflected by attenuation of the vasorelaxant response to metacholine, an endothelium-dependent vasodilator. However, nebivolol-induced vasorelaxation appeared not to be affected by these disease states. We hypothesised that nebivolol, in addition to its $\beta$-blocking and
vasodilator effects, might exert acute endothelium-protective effects, possibly by reducing oxidative stress.

Indeed, very recently several groups were able to show that nebivolol counteracts oxidative stress in animals as well as in humans. For the nonselective β-adrenoceptor antagonist carvedilol, which also displays vasodilator activity presumably via blockade of adrenergic α₁ receptors, such antioxidant activity was already established in the 1980s. ROS are known to provoke lipid peroxidation, DNA oxidation and protein oxidation, which are noxious effects. Furthermore, ROS have shown to play a role in endothelial dysfunction, either by damaging endothelial cell structure or by scavenging of the endothelium-derived relaxing factor NO. In addition, it has been proposed that ROS are important mediators in vascular and cardiac hypertrophy and/or remodelling and that they are involved in vasoconstriction. In cardiovascular diseases, such as hypertension and heart failure, ROS levels are elevated and probably associated with the pathogenesis or progression of the disease. Also in ischemia-reperfusion damage, ROS play an important role. In all of these pathological conditions, it has been shown that carvedilol reduces levels of ROS and thereby prevents ROS-induced damage, for instance endothelial dysfunction. We investigated whether nebivolol, like carvedilol, protects against such ROS-induced endothelial damage. In experiments in the isolated rat aorta (Chapter 5), we could indeed demonstrate that endothelial dysfunction, induced by exogenously generated ROS, was prevented by the presence of nebivolol. Consequently we hypothesized that, if nebivolol acts as a scavenger in this model, it would be consumed by its reaction with ROS. Using a quantitative analytical technique, we could demonstrate that the concentrations of nebivolol were decreased after exposure to ROS, confirming that the nebivolol molecule acts as an antioxidant in this model. Furthermore, we wished to investigate the possible protective effect of nebivolol in ischemia-reperfusion damage. We therefore performed experiments in the isolated rat heart, and administered nebivolol during the reperfusion phase of ischemia-reperfusion (Chapter 6). Nebivolol, like carvedilol, prevented myocardial tissue damage as reflected by a decreased leakage of the enzyme creatine kinase, an indicator of myocardial tissue damage. Furthermore, coronary flow, an indirect parameter for cardiac function, remained stronger when carvedilol or nebivolol were administered. However, in these experiments we could not demonstrate a difference in cardiac contractility, when compared to control preparations.

In order to substantiate the role of ROS in cardiovascular phenomena, we investigated the role of ROS in angiotensin II-induced vasoconstriction. Angiotensin II exerts its
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vasoconstrictive effects on vascular smooth muscle cells via the AT₁ receptor. Since it has been hypothesized that angiotensin II also promotes ROS-generation via this receptor, we studied angiotensin II-induced vasoconstriction in the isolated rat aorta, in the presence of various inhibitors of the pathway leading to the formation of ROS (Chapter 7). The results of these experiments suggest that angiotensin II-induced vasoconstriction is indeed partly mediated by the formation of vasoconstrictor ROS.

Taken together, in the rat aorta, but not in the coronary vascular bed, nebivolol-induced vasorelaxation appears to be NO-dependent and, interestingly, partly mediated by the β₁-adrenergic receptor. Although in hypertension and heart failure, endothelium-dependent vasorelaxant properties of the vasculature appear to be impaired, this beneficial hemodynamical profile of nebivolol persists. Furthermore, we have demonstrated that nebivolol acts as a scavenger of ROS, and thereby prevents ROS-induced endothelial dysfunction and possibly reperfusion damage.