SUMMARY

The disturbance of the normal coronary perfusion of the heart as a result of thrombosis, atherosclerosis and coronary vascular spasms is one of the major direct causes of cardiac ischaemic diseases like angina pectoris and cardiac infarctions. Calcium antagonists, which primarily inhibit the so called slow calcium channels in the plasma membrane, are a relatively new group of drugs used in the treatment of cardiac and coronary diseases. They not only improve the supply of blood to the myocardium (due to their vasodilating effects), but limit the energy need of the heart through a negative inotropic effect and they show some antiarrhythmic activity as well.

In this thesis the effects of the principal classes of calcium antagonists (with nifedipine, verapamil, diltiazem and bepridil as representatives) on the coronary vasculature (vasodilatation) and the myocardium (negative inotropism) are studied in the normal and ischaemic rat heart. A coronary perfusion model of the heart was chosen, as described originally in 1897 by Oscar Langendorff, in which the changes in coronary flow as well as in myocardial contractility can be measured simultaneously. Ischaemia was induced by a complete cessation of the coronary flow.

Large differences in vasoselectivity were found among the various calcium antagonists. For several compounds it was observed that the enantiomers showed a different stereoselectivity towards the coronary vasculature and the myocardium. Furthermore, the kinetics of the effects of these drugs on the coronary vasculature (flow increase) appeared to be much faster than on the heart muscle (negative inotropism); moreover, with the (-)-enantiomers of some verapamil congeners it was found that the wash-out kinetics of the negative inotropic effect were substantially slower than for their optical antipodes. These results indicate that important differences exist between the vascular and myocardial isochannels. The positive inotropic effect, which was observed at low, vasodilating concentrations of calcium antagonists with perfusion under constant pressure conditions was shown not to be a direct (calcium agonistic) activity of the drugs, but a consequence of the Frank Starling principle, induced by the vasodilatation itself (chapters 2, 3 and 4).

In ischaemic conditions (no coronary flow) the depletion of high energy phosphates (ATP, CP) induces a ventricular diastolic contracture, which could be selectively and concentration dependently delayed and inhibited at energy saving (negative inotropic) concentrations of all calcium antagonists studied. Furthermore, a new, protective mechanism was found at the lower, vasodilating concentrations of the drugs, resulting in an improved recovery during the reperfusion phase. Moreover, the protective activities of the calcium antagonists displayed a stereoselectivity which during ischaemia (delay and decrease of the diastolic contracture) was similar to the stereoselectivity of the normal negative inotropic activity, whereas during the reperfusion phase (acceleration of the recovery from the diastolic contracture) a close parallel

was observed with the stereoselectivity of the vasodilating effect of the drugs (chapters 5 and 6). In addition we have found that the calcium agonist BAY K 8644, and especially the laevorotatory enantiomer, aggravated the diastolic contracture and delayed the recovery; these effects were shown in particular at vasoconstricting (reduced oxygen and substrate availability) and positive inotropic (increased energy expenditure) concentrations (chapter 7). Although calcium antagonists until now were considered to be active against the detrimental consequences of ischaemia only when given prophylactically, the results on the protective effects during reperfusion of the (stereoisomers of) vasoselective compounds strongly suggest direct therapeutic effects of this class of drugs as well.

Besides studies with the intact heart, the second lead in this thesis concerns biochemical studies. At the membrane level, calcium transport across the myocardial plasma membrane (sarcolemma) was studied, in particular ATP-dependent Ca^{2+}-transport and Na^{+}/Ca^{2+}-exchange in sarcolemma vesicles. At physiological conditions these processes serve to extrude from the heart the calcium that has entered during the contraction cycle. A differential centrifugation technique was used to obtain purified sarcolemma from rat heart ventricles. Inhibition of these two processes by calcium antagonists was not found to be stereoselective. However, clear differences in drug sensitivity and in the slope of the concentration-inhibition curves between the ATP-dependent Ca^{2+}-transport and the Na^{+}/Ca^{2+}-exchanger were found. The results indicate that the drugs act not only at the level of the slow calcium channel but may also interfere with other (possibly calmodulin-mediated) processes (chapter 8).

Ischaemic situations are usually accompanied by a drop in pH, which alters the degree of protonation and possibly the activity of drugs. However, for bepridil, which at pH 7 is protonated for 17%, the inhibition of the ATP-dependent Ca^{2+}-extrusion and the Na^{+}/Ca^{2+}-exchanger appeared to be independent of the degree of protonation. In contrast, the two calcium transport processes themselves were strongly sensitive to externally applied pH changes (chapter 9), suggesting that in ischaemic conditions the extrusion of accumulated Ca^{2+} may be impaired. This was confirmed by the observation that membranes derived from ischaemically preperfused rat hearts indeed showed a decreased ATP-dependent calcium pumping activity. Pretreatment with verapamil at a relatively low concentration was found to enhance markedly the activity of this Ca^{2+}-pump before the onset of ischaemia and during the reperfusion phase (chapter 10).

In view of these results, it was considered of interest to investigate if the modulation of the vascular and myocardial isochannels in the heart, both in normoxic and ischaemic conditions, could be confirmed at the receptor level. Thus, calcium antagonist binding sites were studied in membrane fractions from normal and ischaemic rat hearts using [^{3}H]-(+)-PN 200-110, a dihydropyridine calcium antagonist selective for the vascular isochannel, and [^{3}H]-(-)-devapamil, a calcium antagonist from the verapamil group, which is more selective for the myocardium. It was found that at negative inotropic (energy saving) concentrations verapamil affords protection against ischaemia-induced changes

in the number of binding sites (Bmax) of [^{3}H]-(+)-PN 200-110. Furthermore, at lower, vasodilating concentrations nifedipine exerted a protective activity selective for the reperfusion phase (chapter 11).

The results from *ex-vivo* and *in-vitro* studies show a clear protective role of calcium antagonists in ischaemia and support the view that these drugs improve the energy balance, the ionic homeostasis and the removal of detrimental agents which are intimately connected to myocardial ischaemic diseases.