Interactions between calcium antagonists and the sympathetic nervous system in various pharmacological models

van der Lee, R.

Citation for published version (APA):
SUMMARY

Chapter 1

The introduction is dealing in detail with the interactions between calcium ions, calcium channels, calcium antagonists and the subsequent effects on vascular smooth muscle. An increase in the cytoplasmic calcium concentration in the vascular smooth muscle cell (VSMC), either by release of calcium ions from intracellular stores or by the massive influx of extracellular calcium ions through calcium channels into the cytoplasm, leads to a contractile response. Calcium antagonists (CA) are able to block the voltage-dependent calcium channels, which mediate the influx of extracellular calcium ions. Several types of these channels are known to exist, and three of these are relevant in cardiovascular medicine: the L-, T- and N-type calcium channels. The most important therapeutic indications for the use of CA are hypertension and angina pectoris. CA appear to be beneficial in these conditions and despite some contradictory reports they are safe when used on long term. Research is ongoing in the areas of left ventricular hypertrophy and atherosclerosis to determine whether CA may be used as therapeutics in these conditions. Many new CA have been developed during the last two decades. The most relevant improvements of the newer CA compared to the older agents are increased vascular selectivity, slower onset of action and prolonged duration of action. For the older CA therapeutic delivery systems have been devised, thus allowing slow release of these compounds and consequently a similar kinetic profile as demonstrated for the newer CA.

Chapter 2

The biphasic contractile responses of rat isolated mesenteric, renal, coronary and basilar small arteries to potassium-induced depolarization were investigated. The tonic phase is assumed to be exclusively the result of L-type calcium channel (LCC) activation, whereas in the generation of the phasic phase T-type calcium channels (TCC) may be involved. In order to evaluate whether TCC blockade has any influence on depolarization-induced contractions the effects of the LCC antagonists nifedipine, diltiazem and verapamil were compared with those of the combined L- and TCC antagonist mibebradil. Small arteries (size 393.6 ± 4.8 μm, n=104) were dissected from the respective organs of male Wistar rats (300-350g) and studied in an isometric wire myograph. The effects of increasing concentrations of the calcium antagonists on repetitive potassium-induced contractions were quantified by means
of cumulative concentration-response curves. A comparison was made with mesenteric vessels of SHR and WKY for nifedipine and mibefradil. Nifedipine was the most potent compound in blocking both the phasic phase (reduction 66-77%) and the tonic phase (IC$_{50}$ = 1.1-5.4 nM). The effect of mibefradil on the phasic response was comparable to that of verapamil and diltiazem. With respect to the tonic response mibefradil was comparable to verapamil (IC$_{50}$ = 19.6-178.9 nM). These findings indicate that the TCC blockade does not contribute to the vasodilator effect of mibefradil under the conditions investigated.

**Chapter 3**

It is rather the rate of the vasodilator effect than its magnitude which determines the triggering of reflex tachycardia associated with dihydropyridine calcium antagonists (DHP-CA). We therefore compared the rate of the vasodilator effects of a series of CA (both DHP and non-DHP) in rat isolated mesenteric artery preparations (size 256 ± 3 μm, length 2 mm) from male Wistar rats (weighing 300-350g.) in an isometric wire myograph. The mean force of the KCl-induced contraction amounted to 2.3 ± 0.1 mN/mm. Potency (given as IC$_{50}$-values), differential time course of action and recovery of the contractile response of the vessels after wash-out were established. These three parameters adhere to the following sequences: (potency) S,S-barnidipine > (S)-lercanidipine > barnidipine HCl > amlodipine > nifedipine, lacidipine > (R)-lercanidipine > verapamil, mibefradil; (differential time course) lacidipine, amlodipine > (S) and (R)-lercanidipine, S,S-barnidipine, barnidipine HCl > mibefradil, verapamil, nifedipine; (recovery) nifedipine > verapamil, S,S-barnidipine, amlodipine > barnidipine, lacidipine > mibefradil, (R)-lercanidipine > (S)-lercanidipine.

In conclusion, S,S-barnidipine proved to be the most potent vasodilator agent; interestingly, barnidipine was 20 times less potent when applied as a racemic mixture. A slow onset of action in DHP is a very important mechanism in preventing reflex tachycardia. For non-DHP (verapamil, mibefradil) reflex tachycardia probably is prevented by a direct effect on the conductive tissue in the myocardium

**Chapter 4**

A slow onset of action for a calcium antagonist (CA) implies a lower degree of sympathetic activation triggered by rapid vasodilation and activation of the baroreceptor reflex. In a previous study we investigated the differential time courses
of various CA in small isolated rat mesenteric arteries. We concluded that the differences observed were most probably related to variations in lipophilicity between the CA studied. Lipophilicity of a chemical entity is quantified by the logarithm of the membrane-partition coefficient (logP). We investigated a possible relationship between the time courses of action of various CA and their lipophilicity, characterised as logP-values. Human small subcutaneous arteries (internal diameter 591 ± 51 m, n=7 for each concentration) were obtained from cosmetic surgery (mamma reduction and abdominoplasty). The vessels were investigated in an isometric wire myograph. The vasodilator effect of the CA was quantified by means of logIC<sub>50</sub>-values, and the onset of the vasodilator effect for each concentration studied was expressed as time to E<sub>eq90</sub>-values (time to reach 90% of the maximal effect. LogIC<sub>50</sub>-values were -8.46 ± 0.09, -8.33 ± 0.25 and -8.72 ± 0.16 for nifedipine, felodipine and (S)-lercanidipine, respectively (n.s.). On average, nifedipine reached time to E<sub>eq90</sub> in 11 ± 1 min. For felodipine and (S)-lercanidipine the corresponding values were 60 ± 11 min and 99 ± 9 min, respectively. The differences between these values were statistically significant (p<0.01). In spite of these differences in the in vitro human vascular model, the three CA are equipotent with regard to their vasodilator effects. The calculated logP-values of the three CA studied were 2.50, 4.46 and 6.88 for nifedipine, felodipine and (S)-lercanidipine, respectively. Linear regression analysis of the correlation between logP-values of the CA tested and their respective values found for time to E<sub>eq90</sub> was highly significant. Apparently, a higher logP-value is correlated with a slower onset of action.

Chapter 5

This study tested whether mibefradil exerts a stronger inhibitory effect than verapamil on sympathetic neurotransmitter release provoked by electrical field stimulation. Tail arteries (diameter 620 ± 9μm) were obtained from male Wistar rats. Ring segments of 2mm length were mounted in an isometric wire myograph. After an appropriate period of equilibration and a priming procedure the vessels were either subjected to electrical field stimulation (EFS; frequency 0.25-4Hz for 30sec) or a concentration-response curve was generated with either noradrenaline (concentration range 0.03-3μM) or ATP (concentration 0.3 mM) which served as baseline parameters. EFS-induced contractions were stable and reproducible and could be blocked by tetrodotoxin (1μM), guanethidine (3μM) and the combination of suramin (0.5mM) and prazosin (3μM). EFS-induced contractions (1 Hz) were almost completely inhibited by 10μM mibefradil (97%) but only partly by 10μM verapamil (73%). Log IC<sub>50</sub>-values were -5.6 for mibefradil and -6.6 for verapamil.
respectively. Calcium antagonists were equipotent in inhibiting noradrenaline (maximum inhibition by mibefradil and verapamil by 70% and 75%, respectively; log IC\textsubscript{50}: -6.5 and -6.7, respectively) and ATP-mediated contractions (maximum inhibition by mibefradil and verapamil by 92% and 97%, respectively; log IC\textsubscript{50}: -6.5 and -7.0, respectively). Consequently mibefradil displays an additional effect on contractions provoked by EFS-induced sympathetic noradrenaline release which cannot be explained by L-type calcium channel blockade. Probably this effect of mibefradil is mediated by the blockade of prejunctional N-type calcium channels, thereby inhibiting sympathetic noradrenaline release. Since activation of the sympathetic nervous system in hypertension is both common and undesirable, a calcium antagonist displaying both L- and N-type calcium channel blocking activities, would have major advantages over calcium antagonists lacking N-type calcium channel blocking activities.

Chapter 6

The T-type prevalent calcium channel blocker mibefradil (MIB) was shown to exert N-type calcium channel blocking properties. Since this particular type of calcium channel is known to be crucially involved in the neuronal release of noradrenaline the possibility of MIB being a sympatholytic drug has been investigated. The sympathoinhibitory action of 3 and 10 \( \mu \text{mol/kg} \) MIB on the tachycardic effect of electrical stimulation of the preganglionic cardioaccelerator nerves in the pithed rat was investigated. Furthermore, the effect of MIB on the dose-response curve of externally applied noradrenaline was studied. To compare the results with a classical L-type calcium channel blocker, the experiments were repeated with 3 and 10 \( \mu \text{mol/kg} \) verapamil (VER). The maximal increase in heart rate in response to electrical nerve stimulation was 96±7 bpm (control, n=6), 70±6 bpm (MIB 3 \( \mu \text{mol/kg}, \) n=8), 57±6 bpm (MIB 10 \( \mu \text{mol/kg}, \) n=5), 93±5 bpm (VER 3 \( \mu \text{mol/kg}, \) n=6) and 46±7 bpm (VER 10 \( \mu \text{mol/kg}, \) n=5). Guanethidine 5 mg/kg i.v blocked the tachycardic response to electrical stimulation at 1.5 and 10 Hz completely. The maximal increase in heart rate in response to noradrenaline was 96±4 bpm (control, n=6), 103±6 (MIB 3 \( \mu \text{mol/kg}, \) n=6), 42±9 bpm (MIB 10 \( \mu \text{mol/kg}, \) n=5), 73±5 bpm (VER 3 \( \mu \text{mol/kg}, \) n=5) and 40±7 bpm (VER 10 \( \mu \text{mol/kg}, \) n=6). Under control conditions and in the presence of 3 \( \mu \text{mol/kg} \) MIB and VER the maximal effect of noradrenaline was reached at 0.1 \( \mu \text{mol/kg} \) whereas in the presence of 10 \( \mu \text{mol/kg} \) MIB and VER it was reached at a dose of 1 \( \mu \text{mol/kg} \). MIB at a dose of 3 \( \mu \text{mol/kg} \) was significantly more effective in reducing the chronotropic response to electrical stimulation when compared to externally applied noradrenaline. For VER the
opposite holds true. These differences were not observed with doses of 10 μmol/kg MIB and VER. It is concluded that MIB, beside its direct effect on cardiac T- and L-type calcium channels reduces the release of noradrenaline from sympathetic nerve endings, most probably by inhibition of presynaptic N-type calcium channels. In the model used this effect is only observable at relatively low concentrations, most probably due to the direct cardiodepressant action of MIB provoked by L-type channel blockade.

Chapter 7

In a previous study we investigated the differential time courses of the vasodilator effect of various calcium antagonists (CA) in small isolated rat mesenteric arteries. We concluded that the differences observed were most probably due to differences in lipophilicity between the CA studied. A measure for lipophilicity is the logarithm of the membrane-partition coefficient (logP). The logP-values of nifedipine and felodipine are 2.50 and 4.46, respectively. It was the aim of the present study to compare the time courses of nifedipine and felodipine effects by means of forearm venous occlusion plethysmography in healthy subjects. Healthy male non-smoking volunteers (age 31 ± 7 yrs, n=14) were studied in the supine position with both forearms stabilized slightly above the level of the heart. Informed consent was obtained prior to each experiment from all subjects. The brachial artery was cannulated; the cannula remained in position throughout the study protocol for direct measurement of blood pressure (MAP 88 ± 3 mmHg) and for the infusion of CA. ECG tracings were made continuously and forearm blood flow (FBF) was measured at 15-second intervals by R-wave triggered venous occlusion plethysmography. The study commenced with the vehicle of either CA (NaCl 0.9% or a PEG400-solution for nifedipine and felodipine, respectively). In four subsequent runs increasing concentrations of CA were studied for 20 min each, at an infusion rate of 0.3 ml/min. During experiments both hands were excluded from the circulation using small wrist cuffs, inflated to at least 40 mmHg over systolic blood pressure. LogIC_{50}-values were -7.46 ± 0.17 and -8.47 ± 0.14 for nifedipine and felodipine, respectively (p<0.01). Averaged K_D-values were 4.3 ± 0.6 and 4.6 ± 0.6 for nifedipine and felodipine, respectively (n.s.). In this model felodipine appears to be a more potent vasodilator than nifedipine. The 100-fold difference in lipophilicity between the two CA tested is apparently not sufficient to cause any difference in K_D-values in the plethysmography experimental setup.