Clinical and Experimental Pharmacology and Physiology (2005) **32**, 708–713

INCREASED VASCULAR SELECTIVITY AND PROLONGED PHARMACOLOGICAL EFFICACY OF THE L-TYPE Ca^{2+} CHANNEL ANTAGONIST LERCANIDIPINE IN HUMAN CARDIOVASCULAR TISSUE

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SUMMARY

1. The present study investigates the vasoselectivity of lercanidipine (LER), a 1,4-dihydropyridine calcium channel blocker, compared with amlodipine (AML) and nifedipine (NIF) in human cardiovascular tissue. Experiments were performed either in human left ventricular failing myocardium (orthotopic heart transplants) or in isolated right atrial trabeculae and isolated vessel preparations of arteria mammaria obtained from patients undergoing aortocoronary bypass operation.

2. The obtained rank order for the L-type Ca^{2+} channel affinity in human tissue was LER > NIF ≥ AML. Lercanidipine had the lowest negative inotropic efficacy (1 μmol/L LER: 60.3% basal < AML: 79.1% basal < NIF: 92.4 basal) and potency (IC_{50} NIF: 3.5 nmol/L < AML: 48 nmol/L < Ler: 127 nmol/L) in right atrial trabeculae.

3. The vasorelaxant potency of LER (IC_{50} 0.5 nmol/L) and AML (IC_{50} 0.8 nmol/L) was similar and significantly increased compared with that of NIF (IC_{50} 5.9 nmol/L) in arteria mammaria preparations of the very same patients.

4. The following rank order was obtained for vasoselectivity: LER (260) < AML (60) < NIF (0.6).

5. The pharmacological effects of LER and AML were still present 2 h after drug washout.

3. Lercanidipine is characterized by a high vasoselectivity and a prolonged interaction with the L-type calcium channel in human cardiovascular tissue This may be advantageous, especially in the treatment of patients with arterial hypertension.

Key words: amlodipine, lercanidipine, nifedipine vasoselectivity.

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Received 16 September 2004; revision 12 April 2005; accepted 17 April 2005.

INTRODUCTION

Various clinical trials have demonstrated the efficacy and safety of calcium channel blockers in the treatment of hypertension.[1–4] The so-called 'first-generation' calcium channel blockers, like nifedipine, are characterized by a poor vascular selectivity that leads to inotropic impairment, especially in situations of an already decreased cardiac function, or in situations where a combination with a beta-blocker is desirable for the treatment of the patient.[5–8] Furthermore, the fast onset and short duration of the pharmacological effects of the first-generation calcium channel blockers results in a compensatory activation of the sympathetic nervous system,[9] which may unfavourably influence the outcome of the patients.

The second-generation calcium channel blockers, like nicardipine, are characterized by a higher vascular selectivity than the first-generation compounds, thus providing a therapeutic advantage in the treatment of heart failure patients with an already compromised cardiac function.[10–12] However, as observed for the first-generation calcium channel blockers,[13] the short duration of action requires dosing two or three times a day for the optimal treatment of the patients; a fact that may reduce patient compliance. Thus, a high vascular selectivity and a long-lasting pharmacological action are desirable attributes of new calcium channel blockers.

Lercanidipine (methyl 1,1-dimethyl-2-[*N*-(3,3-diphenylpropyl)-*N*-methylamino]ethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride) is a novel third-generation calcium channel blocker with high vascular selectivity and a gradual onset and long duration of action, as shown in the cardiovascular tissue of rabbits and rats.[14,15] However, it is well known that the effect of calcium channel blockers differs markedly between the tissues of different species, as well as between different tissues of the same species.[16–18] Studies investigating the vasoselectivity of lercanidipine in human tissue are still lacking. Therefore, the present study investigated *in vitro* the calcium channel antagonistic effects of lercanidipine on human myocardium and arteries compared with those of amlodipine, another third-generation calcium channel blocker, and nifedipine. To exclude interference by haemodynamic factors, such as changes in preload, afterload or heart frequency, the drugs tested were examined in electrically driven multicellular heart muscle strips or

isolated vessel rings. Furthermore, the binding profile of the three calcium channel blockers to the dihydropyridine binding site of the L-type Ca^{2+} channel was determined in human myocardium.

METHODS

Patients and materials

Human myocardium, from terminally failing human hearts (New York Heart Association functional class IV), was obtained from patients after heart transplantation ($n = 6$, three males and three females; mean (±SD) age 47 ± 8 years). Patient characteristics are given in Table 1.

Human right atrial myocardium and small sections of the arteria mammaria were obtained from patients undergoing aortocoronary bypass operations without clinical signs of cardiac failure, as measured by echocardiography and heart catheterization (normal ejection fraction, end-diastolic volume and stroke volume; $n = 41$, 30 males and 11 females; mean age 63 ± 2 years). Medical treatment of the patients consisted of diuretics, nitrates, β-adrenoceptor antagonists, angiotensin-converting enzyme (ACE) inhibitors and cardiac glycosides. The patients included in the study had not received calcium channel blockers for at least 7 days before surgery. Drugs used for general anaesthesia were flunitrazepam, fentanyl and pancuronium bromide with isofluran. Cardiac surgery was performed on cardiopulmonary bypass with cardioplegic arrest during hypothermia. Immediately after excision, the heart tissue was placed in ice-cold Bretschneider solution (composition (in mmol/L): NaCl 15; KCl 10; $MgCl_2$ 4; histidine 180; tryptophane 2; manitol 30; potassium dihydrogen oxoglutarate 1). Sections of arteria mammaria were placed immediately after excision in ice-cold Tyrode's solution (composition (in mmol/L): NaCl 119.8; KCl 5.4; $MgCl_2$ 1.05; $CaCl_2$ 1.8; $NaHCO_3^-$ 22.6; $NaHPO_4$ 0.42; glucose 5.05; ascorbic acid 0.28; disodium EDTA 0.05, pH 7.4). Transportation to the laboratory took approximately 5 min.

Isolated human arteria mammaria rings

Vascular experiments were performed in isolated vessel rings of human arteria mammaria. Connective tissue and fat were carefully trimmed away and ring segments (length 4–6 mm) were dissected. The artery rings were placed immediately in an organ bath containing Tyrode's solution, as described above, at 37°C and gassed continuously with 95% O_2 and 5% CO_2. Vessels were attached to two high-grade stainless-steel pins. Isometric force of contraction was measured with an inductive force transducer (W Fleck, Mainz, Germany) that was connected to a Gould recorder (Gould, Cleveland, OH, USA). Artery rings were passively loaded with 2 g resting tension and were allowed to equilibrate for at least 90 min until complete mechanical stabilization. During this time, the Tyrode's bathing solution was changed every 30 min. At the beginning of the experiment, artery rings were placed at their optimal length for tension development. This was accomplished by gradually increasing the resting tension until the reaction to 100 mmol/L KCl was optimized. The arteria mammaria rings were left at that tension throughout the experiment. To investigate the vasodilatory effect of the different compounds, rings were precontracted by prostaglandin $F_{2\alpha}$ (0.3 μmol/L). Afterwards, a single concentration of nifedipine, amlodipine or lercanidipine was added to the bathing solution for a period of 3 h. The vasodilatation was measured over 3 h owing to the slow and prolonged onset of the vasodilator action of amlodipine and lercanidipine, as described previously.[14,15] The vasodilating effects of the calcium channel blockers tested were compared with the relaxation of the vessel induced by papaverine (100 μmol/L). All experiments were performed under protection from light. Experiments were performed as described previously.[19,20]

To investigate the duration of the vasodilator activity of lercanidipine and amlodipine, the recovery of the contractile response to 80 mmol/L KCl after drug washout was tested (referred to as basal contraction; 100%). After 30 min stable contraction, the compounds to be tested were added to the organ bath. After 60 min incubation time, the medium was replaced by drug-free Tyrode's solution. After drug washout, contractions were induced by the addition of KCl (80 mmol/L) hourly for 3 h. After each KCl-induced contraction, the Tyrode's solution was changed. Experiments were performed, with some modification, as described by van der Lee *et al.*[21]

Contraction experiments

Because atrial myocardium is more sensitive to changes in the intracellular Ca^{2+} concentration, the negative inotropic effects of nifedipine, amlodipine and lercanidipine were investigated in isolated, electrically driven (1 Hz) right atrial muscle strips.[22,23] Preparation of the muscle strips was performed in ice-cold Bretschneider solution (see above for composition of the solution). During the preparation of the muscle strips, the connective tissue was carefully trimmed away and muscle strips of uniform size (thickness < 0.5 mm, length 3–7 mm) with muscle fibres running approximately parallel to the length of the muscle strips were prepared. The isolated muscle strips were attached to two metal pins in a 75 mL organ bath containing Tyrode's solution (see above) at 37°C and gassed continuously with a mixture of 95% O_2 and 5% CO_2. The force of contraction was measured with a force transducer (W Fleck), which was attached to a Gould chart recorder. Muscle contractions were induced by electrical stimulation with 1 Hz rectangular pulses of 5 msec duration (stimulator model SD9; Grass Instrument, Quincy, MA, USA). The voltage was above 20% threshold. Muscle strips were passively loaded with 1 g resting tension and allowed to equilibrate at least for 90 min in a drug-free solution until complete mechanical stabilization. The bathing solution was changed at least twice during this equilibration period. When the developed force reached a steady state, one concentration of nifedipine, amlodipine or lercanidipine was added into one bathing solution. The force of contraction was evaluated over 3 h owing to the slow and prolonged onset of the negative inotropic effect from amlodipine and lercanidipine.[14,15] Control experiments were performed in drug-free Tyrode's solution. All experiments were performed under protection from light. Experiments were performed as described previously.[20]

Binding studies

Membrane preparation

Binding experiments were performed to measure the affinity of nifedipine, amlodipine and lercanidipine to the dihydropyridine subunit of the L-type

Table 1 Clinical characteristics of patients with dilated cardiomyopathy

Patient no.	Sex	Age (years)	MRAP (mmHg)	LVEDP (mmHg)	EF (%)	CI (L/min per m^2)	AT_1 antagonist	ACE inhibitor	Digitalis	Nitrates
1	F	32	–	–	–	–		✓	✓	
2	F	66	7	9	28	2.7	✓		✓	
3	F	17	8	10	–	2.1	✓		✓	
4	M	59	7	–	53	3		✓	✓	✓
5	M	41	5	9	20	3		✓	✓	
6	M	65	8	15	39	–		✓	✓	

EF, ejection fraction; MRAP, mean right atrial arterial pressure; LVEDP, left ventricular end-diastolic pressure; CI, cardiac index.

Ca^{2+} channel in human myocardium. The tissue was chilled in 15 mL ice-cold homogenization buffer (composition (in mmol/L): Tris HCl 40; EDTA 1; dithiotreitol 1, pH 8.0). Connective tissue and fat were trimmed away and the myocardial tissue was minced with scissors. The minced tissue was homogenized with a motor-driven glass–Teflon potter for at least 3 min and afterwards by hand for 1 min. The homogenates were centrifuged at 480 *g* for 15 min at 4°C and the supernatants were diluted with an equal volume of 1 mol/L KCl and stored on ice for 10 min. The supernatants were centrifuged at 100 000 *g* for 45 min and the pellets were resuspended in ice-cold incubation buffer (composition (in mmol/L): Tris HCl 50; $MgCl_2$ 10, pH 7.4) and centrifuged again at 100 000 *g* for 45 min. The final pellets were resuspended in incubation buffer. These final solutions had a protein concentration of 2–6 mg/mL, as determined by Bradford measurements, and were stored at –80°C.

Radioligand binding assay

The radiolabelled ligand used in the present study was [^{3}H]-PN-200-110 because of its high affinity for the dihydropyridine receptor subunit of the L-type Ca^{2+} channel.[24] Membrane solutions were incubated with nifedipine, amlodipine or lercanidipine at pH 7.4 for 1 h at 37°C. The incubation was terminated after 1 h by rapid vacuum filtration with a cell harvester (M125 Brendel Labortechnik, Asbach, Germany) through Whatman GF/C filters (Whatman International, Maidstone, UK). The filters were washed three times with 6 mL ice-cold incubation buffer. After drying, the radioactivity in the filters was measured by liquid scintillation counting with a scintillation counter (model 1272; LBK Wallac, Clinigamma, Germany). Non-specific binding was evaluated using 1 μmol/L nifedipine. The specific binding of the radioligand was calculated by subtracting the non-specific binding from total binding. Experiments were performed as described previously.[25]

Materials

Nifedipine was generously provided by Bayer AG (Leverkusen, Germany), amlodipine was provided by Pfizer (Karlsruhe, Germany) and lercanidipine was from UCB Pharma (Kerpen, Germany). All compounds were of analytical grade or the best grade available commercially. The [^{3}H]-PN-200-110 was obtained from Amersham Biosiences (Braunschweig, Germany). Drugs were dissolved in dimethylsulphoxide (DMSO). The final concentration of DMSO in the organ bath was never higher than 0.05%. All substances were protected from light.

Statistical analysis

The results are presented as the mean±SEM. To determinate the statistical significance of differences, Student's *t*-test or one-way analysis of variance (ANOVA) was used, including a Bonferoni's post test. $P < 0.05$ was set as the level of statistical significance. The concentration of a drug required to decrease basal force of contraction of isolated human right atrial muscle strips by 50% or to achieve a 50% vasorelaxation (IC_{50} values) was calculated using GraphPad Prism (GraphPad, San Diego, CA, USA).

RESULTS

Binding to the dihydropyridine L-type Ca^{2+} channel

Figure 1 shows the competition curves obtained by inhibition of specific [^{3}H]-PN-200-110 binding with nifedipine, amlodipine and lercanidipine in crude membrane preparations of human hearts (for $n = 3$–4 in triplicate for each drug). Lercanidipine had the highest affinity for the dihydropyridine-binding site of the L-type Ca^{2+} channel. The obtained rank order for the L-type Ca^{2+} channel affinity was lercanidipine (K_i 2.2 /nmol; 95% confidence interval (CI) 1.6–3.2 /nmol) > nifedipine (K_i 8.1 /nmol; 95% CI 5.6–17 / nmol) ≥ amlodipine (K_i 10.9 /nmol; 95% CI 7.2–16 /nmol).

Effect on vascular tone

The concentration-dependent effects of nifedipine, amlodipine and lercanidipine on isolated vessel rings from human arteria mammaria preparations are summarized in Fig. 2. All Ca^{2+} channel blockers concentration-dependently (0.0001–10 μmol/L) reduced vascular tension. At the highest concentration used (10 μmol/L), all three calcium channel blockers showed a similar vasodilatory efficacy (80.1 ± 2, 78.5 ± 3.2 and 82.8 ± 2.7% of the vasodilatory effect of 100 μmol/L papaverine (%PAP) for lercanidipine, amlodipine and nifedipine, respectively). The rank order of vasorelaxant potency as judged by the IC_{50} values was nifedipine (5.9 nmol/L; 95% CI 0.8–29.0 nmol/L) < amlodipine (0.8 nmol/L; 95% CI 0.07–8.7 nmol/L) = lercanidipine (0.49 nmol/L; 95% CI 0.06–4.2 nmol/L).

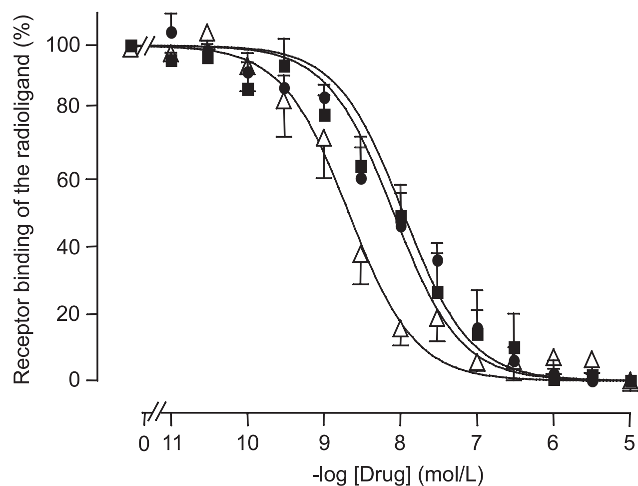

Fig. 1 Inhibition curves of specific [^{3}H]-PN-200-110 binding in human heart tissue homogenates by nifedipine (■), amlodipine (●) and lercanidipine (△) demonstrating L-type Ca^{2+} channel selectivity in human right atrial myocardium. Data are the mean±SEM of three to four different experiments (triple measurements for each experiment).

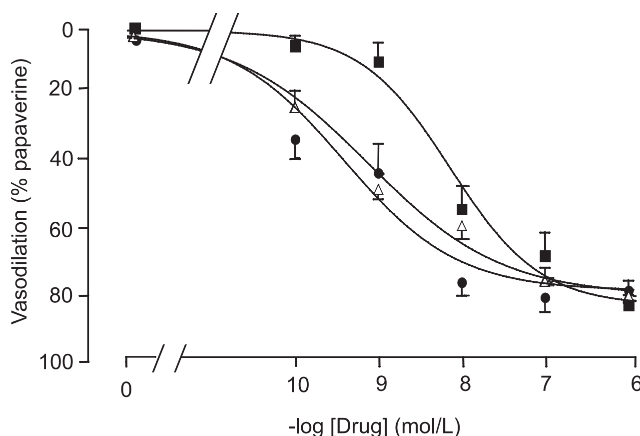

Fig. 2 Vasodilator effects of nifedipine (■), amlodipine (●) and lercanidipine (△) in isolated vessel rings obtained from human arteria mammaria preparations. Isolated vessel rings were precontracted by 0.3 μmol/L prostaglandin $F_{2\alpha}$. Vasodilation is given as a percentage decrease of the maximal value (i.e. the vasodilator effect of 100 μmol/L papaverine). Data are the mean±SEM of six preparations for each concentration.

Effects of drug washout on human arteria mammaria vessels

To investigate the temporal characteristics of the vasodilating effects of lercanidipine, amlodipine and nifedipine, the recovery of contractility after drug washout was tested in isolated human arteria mammaria vessel rings. For these experiments, the concentration of calcium antagonists used was similar to the estimated IC_{50} value for vasodilation (10 nmol/L nifedipine; 1 nmol/L amlodipine and lercanidipine). The results are shown in Fig. 3. Although the vasodilation produced by 10 nmol/L nifedipine was significantly greater than produced by 1 nmol/L amlodipine and lercanidipine, the recovery of contraction in the arteries incubated with nifedipine was partially reversed and almost complete by 2 h after drug wash out, indicating a fast drug offset. There was also a slight recovery of contractility by vessel rings incubated with amlodipine. In contrast, vessel preparations incubated with lercanidipine showed a slow and constant decrease in contractility, even 2 h after drug wash out, indicating that the pharmacological effects of lercanidipine are maintained after stopping external drug support.

Effect on human right atrial myocardium

The negative inotropic effects of lercanidipine, amlodipine and nifedipine on the electrically stimulated right atrial myocardium are presented in Fig. 4. All compounds tested induced a concentration-dependent (0.0001–10 μmol/L) negative inotropic effect on human isolated, electrically stimulated right atrial muscle strips. At the highest concentration tested (10 μmol/L), the rank order of negative inotropic potency was nifedipine (92.1 ± 1.9% basal) > amlodipine (79.3 ± 6.9% basal) > lercanidipine (60% ± 4.2% basal). The rank order of the negative inotropic effect as judged by the IC_{50} values was nifedipine (3.5 nmol/L; 95% CI 1.7–7.2 nmol/L) > amlodipine (48 nmol/L; 95% CI 7.7–298 nmol/L) > lercanidipine (127 nmol/L; 95% CI 5.9–274.5 nmol/L).

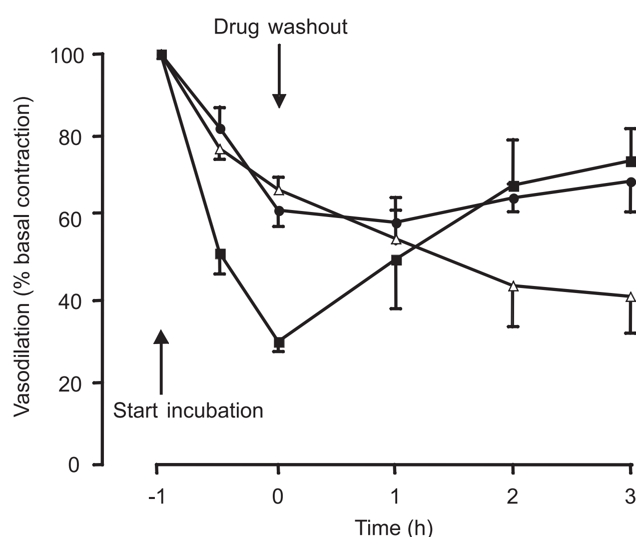

Fig. 3 Recovery of contractile response to 80 mmol/L KCl after drug washout. Human arteria mammaria rings were incubated with 10 nmol/L nifedipine (■), 1 nmol/L amlodipine (●) and 1 nmol/L lercanidipine (△) for 1 h before drug washout ($n = 4$ for each drug). Vasodilation is expressed as a percentage of the basal contraction. The start of the incubation was at −1 h; drug washout was at 0 h. Data are the mean±SEM of K^+ (80 mmol/L)-induced contractions after 1, 2 and 3 h washout.

Vasoselectivity

In the present study, the concentration necessary for a 50% relaxation of the arteria mammaria vessels was compared with the concentration needed to reduce basal force of contraction in right atrial muscle strips by 50%. The ratio of these values is the heart/vessel selectivity index. In comparison with its 50% vasodilatory effect, a 260-fold higher concentration of lercanidipine was necessary to reduce basal force of contraction by 50%. The corresponding value for amlodipine was 60 and the heart/vessel selectivity index of nifedipine was < 1. Thus, lercanidipine has a higher vasoselectivity in human cardiovascular tissue compared with nifedipine and amlodipine.

DISCUSSION

The present study investigated the vasoselectivity of the third-generation calcium channel blocker lercanidipine in comparison with amlodipine and nifedipine. Experiments were performed in intact vessel preparations, as well as in intact trabeculae. Lercanidipine was the calcium channel blocker with the highest L-type Ca^{2+} channel affinity in human tissue. Regarding the drug offset effect, only lercanidipine showed vasodilatory effects even after drug wash out. Despite similar vasodilatory properties of lercanidipine and amlodipine, the vasoselectivity of lercanidipine was significantly increased compared with amlodipine.

The results of the radioligand binding studies demonstrate that lercanidipine has a high affinity for the L-type Ca^{2+} channel in human myocardium. These results are in line with binding studies performed in brain and heart tissues of rat.[14] The high affinity of lercanidipine for the L-type Ca^{2+} channel has been attributed to a competitive binding of lercanidipine with the 1,4-dihydropyridine subunit of the L-type Ca^{2+} channel.[14] Despite this high L-type Ca^{2+} channel affinity, the negative inotropic effect of lercanidipine was

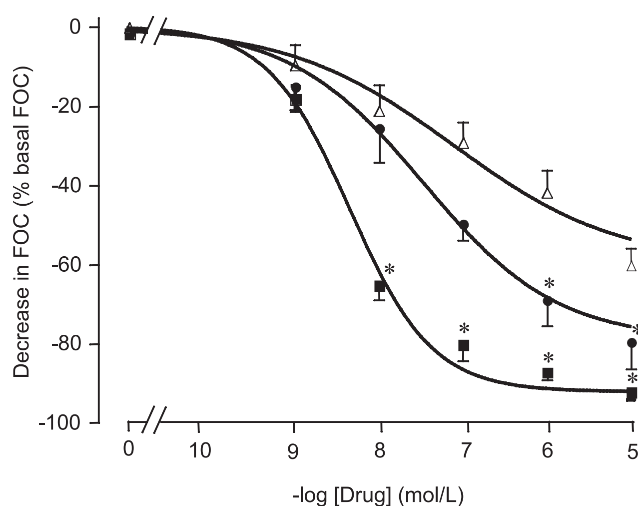

Fig. 4 Negative inotropic effects of the Ca^{2+} channel blockers nifedipine (■), amlodipine (●) and lercanidipine (△) on isolated human right atrial muscle strips. Decreases in the force of contraction (FOC) are compared with the basal force of contraction. *$P < 0.05$ compared with lercanidipine.

much less pronounced compared with that of amlodipine and nifedipine, as shown by the present study in human myocardium, as well as in rat ventricular muscle preparations.[14]

The L-type Ca^{2+} channels exist in different states depending on the membrane potential of the cells. At the most negative diastolic membrane potential, being –80 mV, which is typically for cardiac muscle, virtually all channels are in the low-affinity resting state. This conformation is not or only very weakly susceptible to 1,4-dihydropyridine blockade.[26] In contrast, dihydropyridines bind very tightly to the inactivated, high-affinity state of the calcium channels, which is predominant at depolarized potentials of –10 to 0 mV.[26] Thus, the efficacy of the Ca^{2+} channel blocking effects of 1,4-dihydropyridines may vary during the excitation–contraction cycle of the cardiomyocytes, being most effective during peak systole.

Because vascular muscle has a much less negative resting potential (–50 to –60 mV) than cardiac muscle, which has the most negative diastolic membrane potential of –80 mV,[27,28] it may be assumed that a larger fraction of the vascular Ca^{2+} channels may be in the high-affinity inactivated state, susceptible to high-potency blockade by lercanidipine. This means that the explanation for the high vasoselectivity of lercanidipine in comparison with other 1,4-dihydropyridines may be due to differences in the relative proportions of the different states of the L-type Ca^{2+} channels in vascular and cardiac muscle.[13]

Because the binding experiments are performed using disrupted membrane fragments, where the membrane potential would be near 0 mV and all the Ca^{2+} channels in the high-affinity inactivated state, the affinity of the compounds for the calcium channel have been evaluated under the most favourable conditions.[14] A good correlation has been reported between vascular and cardiac selectivity (evaluated as the ratio of IC_{50} in rat papillary muscle and IC_{50} in rat tail artery) and the index of the voltage-dependent binding (ratio between the affinity for the calcium channel in rat cardiomyocytes under polarized and depolarized conditions[29]), supporting the idea that voltage dependence is probably the most important determinant for the vascular selectivity of the 1,4 dihydropyridines.

The slow onset and the long duration of the antihypertensive effect of lercanidipine (even after wash out) may be explained by its high lipid solubility (a partition coefficient (log P) of 6.0 was determined[28]), which should allow the molecule to accumulate inside the smooth muscle cell membrane. From these lipid depots, lercanidipine may be released continuously and slowly, subsequently reaching L-type Ca^{2+} channels.[30,31] Such accumulation could be the reason for the prolonged calcium antagonistic activity of lercanidipine,[14] which allows good control of blood pressure over 24 h by means of a single dose.[32] Owing to the slow onset of action, sympathetic activation and reflex tachycardia do not occur. In line with this suggestion, it has been shown previously for different pharmaceutical formulations of nifedipine that reflex tachycardia is triggered by the rate of the vasodilator effect rather than its magnitude.[33] The slow release of lercanidipine from the lipid depot in the cell membrane also explains the long duration of action of this lipophilic calcium channel blocker

Amlodipine is less lipophilic and possesses a tertiary amino side-chain with pK_A of 8.6; at pH 7.4, this side-chain is protonized (and, thus, positively charged) and may, thus, interact electrostatically with the negatively charged region of the phospholipid head group of the membrane.[34] This interaction may slow the diffusion of the molecule through the membrane to its receptor site, in addition to keeping the molecule at its receptor site once it is bound.[20] Accordingly, these structural characteristics of amlodipine may account for its slow onset and offset of action and dissociation kinetics.[35] In the present study, amlodipine showed a slow drug offset in contrast with lercanidipine. However, it has to be mentioned that the offset characteristics of amlodipine have been reported to be concentration dependent.[36]

In conclusion, the present study provides evidence that suggests that the Ca^{2+} channel blocker lercanidipine has a high vasoselectivity in human cardiovascular tissue. The high vasoselectivity of lercanidipine may be of advantage in heart failure patients with an already depressed cardiac function and supports a high efficacy over time to reduce blood pressure in arterial hypertension.

ACKNOWLEDGEMENTS

The authors are indebted to all their colleagues of the Department of the Cardiothoracic Surgery of the University of Cologne (Professor Dr RE de Vivie, Director) for providing us with human samples. This work was supported by UCB Pharma, Kerpen, Germany. The authors thank Mrs Esra Koröglu, Katja Rössler and Kerstin Schenk for their excellent technical help. This work contains parts of the doctoral thesis of TG.

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