J. Pharm. Pharmacol. 1999, 51: 709–714
Received December 7, 1998
Accepted February 5, 1999

Vascular-selective Effect of Lercanidipine and other 1,4-Dihydropyridines in Isolated Rabbit Tissues

P. ANGELICO, L. GUARNERI, A. LEONARDI AND R. TESTA

Pharmaceutical R & D Division, Recordati S.p.A., Milano, Italy

Abstract

The aim of this study was to characterize the in-vitro vasoselectivity of lercanidipine (in comparison with lacidipine, amlodipine, nitrendipine and felodipine) by evaluating its functional calcium antagonistic activity on rabbit vascular (aorta) and cardiac tissues (heart ventricle).

Although incubation with all the compounds tested elicited a concentration-dependent relaxant effect on vascular tissue precontracted with KCl (80 mM), 50% relaxation was reached at different times for each concentration and drug tested. At 10 nM concentration 50% relaxation was reached after 210 min with lercanidipine, 278 min with amlodipine, 135 min with lacidipine, 75 min with nitrendipine and 70 min with felodipine. The onset of the effect was, therefore, similar for lercanidipine, amlodipine and lacidipine, but faster for nitrendipine and felodipine. Similarly, all the compounds tested concentration-dependently reduced the force of cardiac contraction (negative inotropic activity). In this model, the time needed to reach 50% reduction in contractile force was also concentration-dependent, and the ranking order of the speed of onset of the effect (evaluated as the ratio of the IC50 values (the concentrations inhibiting contraction by 50%) calculated after 1 and 4 h incubation) was lacidipine (3·8) > amlodipine (9·6) > felodipine (39) > lercanidipine (68) = nitrendipine (89). The vasoselectivity, expressed as the ratio of the IC50 values obtained on cardiac and vascular tissue, were (for 4 h incubation) 730, 193, 95, 6 and 3 for lercanidipine, lacidipine, amlodipine, felodipine and nitrendipine, respectively, showing that lercanidipine is the most vasoselective of the calcium-antagonists tested.

The results show that lercanidipine reduces the inotropic force of the rabbit heart to a lesser extent than do other calcium antagonists, and that this drug had the best heart/vessel selectivity index among the compounds tested at all the times tested.

Lercanidipine (Zanidip) is a 1,4-dihydropyridine-type calcium antagonist, synthesized by the Pharmaceutical R&D Division of Recordati (Leonardi et al 1998) and recently approved for treatment of hypertension in several European countries.

An extensive review of its pharmacodynamic, pharmacokinetic, toxicological and clinical properties has recently been published (Testa et al 1997). These studies included in-vitro evidence of the enhanced vascular selectivity of lercanidipine in comparison with nifedipine, nicardipine, nitrendipine and felodipine (Guarneri et al 1996, 1997). Vasoselectivity was evaluated by comparing the depressant effects of these compounds on electrically driven rabbit-heart ventricular strips or guinea-pig right atria with their potency in relaxing KCl-contracted rat aorta.

Correspondence: P. Angelico, Pharmaceutical R & D Division, Recordati S.p.A., Via M. Civitali 1-20148, Milano, Italy.

The sensitivity of extracellular calcium influx to antagonists not only varies among different tissues within a species (Mecca & Love 1992; Magnon et al 1995) but also varies widely on the same tissue from different species (Lederballe Pedersen 1981), indicating that the vasoselectivity index of compounds has more validity if it is calculated by using vascular tissues and heart preparations taken from the same species (Magnon et al 1995).

The aim of this study was, therefore, better characterization of the in-vitro vasoselectivity of lercanidipine by evaluating its functional calcium antagonistic activity on vascular (aorta) and cardiac tissue (heart ventricle strip) taken from the same species, the rabbit.

It has been reported that the pharmacodynamic and pharmacokinetic properties of calcium antagonists are also related to their kinetic and equilibrium interactions with membranes (Herbette et al 1991). The membrane-partition coefficient of lercanidipine in cardiac model membranes was similar to that of lacidipine, higher than that of amlodipine and markedly higher than that of nitrendipine (Herbette et al 1997).

Amlodipine and lacidipine were therefore used as reference substances, as were nitrendipine and felodipine. Some of these data have already been presented in abstract form (Angelico et al 1998).

Materials and Methods

Animals

Male New Zealand White rabbits, 2·5–3·5 kg, were housed with free access to food and water and maintained on a forced 12-h light–dark cycle at 22–24°C for at least one week before experiments were performed.

The animals were handled according to internationally accepted principles for care of laboratory animals (EEC Council Directive 86/609, OJL358, 1, December 12, 1987).

Drugs

Lercanidipine hydrochloride, felodipine and nitrendipine were synthesized in our chemical laboratories, amlodipine besilate and lacidipine were isolated from their pharmaceutical forms, and all the other drugs or materials were obtained from commercial sources. In the experiments the drugs tested were dissolved in aqueous dimethylformamide (1% v/v) containing Tween 80 (1% v/v) at 1×10^{-3} M stock concentration, and further diluted with distilled water as necessary.

Functional Ca^{2+}-antagonistic activity on vascular tissue

Rabbits were killed by cervical dislocation and the thorax was opened along the midline. The thoracic aorta was removed and eight rings per aorta, 4–5 mm wide (approx.), were cut and suspended between two stainless steel wires in a 20-mL organ bath containing Krebs solution (mM: NaCl 112, KCl 5, KH_2PO_4 1, $MgSO_4$ 1·2, $NaHCO_3$ 12, glucose 11·1, $CaCl_2$ 2·5), equilibrated at 37°C with 95% O_2 and 5% CO_2. The rings were connected to isometric transducers (DY10, Basile, Italy) and loaded with 2 g under isometric conditions. After 60 min stabilization contractions were induced by adding KCl (final concentration in the bath, 80 mM) and recorded by means of a two-channel recorder (Gemini 7070, Basile, Italy). When the tonic component of the contraction was constant (after approx. 15 min), vehicle or one single concentration of each compound was added to the bath. The relaxant effect was evaluated after 1, 2, 3 and 4 h contact time.

Functional Ca^{2+}-antagonistic activity on cardiac tissue (negative inotropic activity)

Rabbits were killed as above and the heart was excised quickly and washed with oxygenated Krebs solution. The wall of the right ventricle was cut and further dissected into strips measuring 2 mm × 20 mm (approx.). The strips were transferred to a 50-mL organ bath containing Krebs solution (mM: NaCl 111·2, KCl 4·7, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25, glucose 11·5, $CaCl_2$ 3·2), equilibrated at 37°C with 95% O_2 and 5% CO_2. Each strip was hooked to a needle-shaped platinum electrode for electrical stimulation and to an isometric transducer as described above. Strips were loaded with 1 g resting tension and were left to equilibrate for 45 min. Contractions were induced by electrical stimulation (BM-ST3 stimulator, Biomedica Mangoni, Italy) at a frequency of 2·5 Hz, a potential of 10–30 V and a width of 0·5–2 ms. When the developed force was constant, the basal inotropic activity was measured. Then, vehicle or one single concentration of each tested compound was added to the bath and the changes in contractile activity were observed for up to 4 h. The negative inotropic effect was evaluated after 1, 2, 3 and 4 h contact time.

Data analysis

Experimental groups consisted of at least three preparations taken from different animals for each concentration tested. The relaxant effect was evaluated after 1, 2, 3 and 4 h contact time and expressed as percentage relaxation in comparison with the predrug maximum contraction, taken as 100%. The negative inotropic effect was evaluated at the same times as above and expressed as percentage reduction of the developed tension compared with predrug basal values, taken as 100%. Data are expressed as mean ± s.e.m.

The time–concentration response curves were analysed by use of a program (Allfit, from N.I.H.) for non-linear fitting of the logistic equation as described by De Lean et al (1978), using the percentage relaxation or inhibition of the contractility

of ventricular strips (% of basal value) obtained at each concentration.

The function used was:

$$\text{Response} = E_{max} + (E_{min} - E_{max})/(1 + X/(X50)^{-s}) \quad (1)$$

where Response was the percentage relaxation (aorta) or the negative inotropic effect, X was the time to reach the corresponding effect or the concentration inducing the observed effect at a fixed time, E_{max} and E_{min} were the maximum and minimum responses observable (always forced to 100% and 0%, respectively), X50 was the mid point location parameter and s was the slope of the curve. X50 values and their 95% confidence limits were computed. Linear regression analysis was performed by use of a standard package (Lotus).

Results and Discussion

The endothelium in the vascular preparations used in this study was not preserved, as confirmed by the lack of relaxing effect of acetylcholine on some KCl-precontracted aortic rings (data not shown). Because the effects of calcium antagonists are not endothelium-dependent (Jayakody et al 1987), lack of intact endothelium should not influence the reported results.

Functional Ca^{2+}-antagonistic activity on vascular tissue

In control preparations the addition of the vehicle used for solubilization of the compounds tested caused a negligible fall in tension of rabbit aorta. Incubation with all tested compounds induced a concentration-dependent relaxant effect on precontracted vascular tissue. An example of this, the relaxant effect of different concentrations of lercanidipine and nitrendipine, is shown in Figure 1.

Although the effects of both drugs were concentration-dependent, 50% relaxation was reached at different times for each concentration and drug tested. At 10 nM concentration, for example, nitrendipine needed 75 min to reduce by 50% the K^+-induced contraction of the vessel, whereas the same concentration of lercanidipine required 210 min. By increasing or reducing the concentrations of both compounds it was shown that the time needed to reach 50% of the effect changed linearly with concentration (Figure 2). The slopes of the curves in Figure 2 represent, therefore, the speed of onset of the effect as a function of the concentrations tested. A steeper curve indicates a more rapid onset of effect.

The time taken to reach 50% relaxation of rabbit aorta rings for all tested compounds and concentrations is shown in Table 1, together with the slopes of the curves correlating concentration and time. The onset of the effect was similar for lercanidipine, amlodipine and lacidipine, but faster for

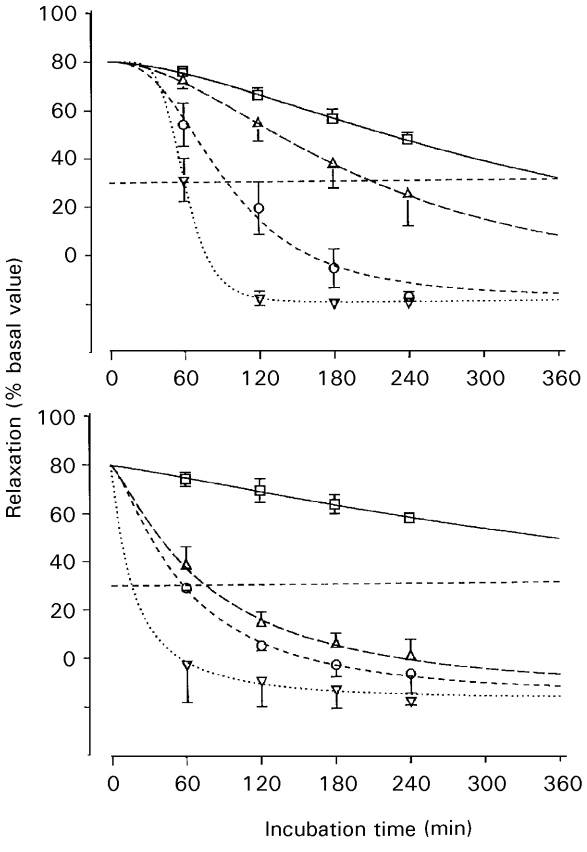

Figure 1. Relaxation of rabbit aorta rings precontracted by KCl (final bath concentration 80 mM), after incubation with different concentrations (□, 1; △, 10; ○, 100; ▽, 1000 nM) of lercanidipine (upper) or nitrendipine (lower).

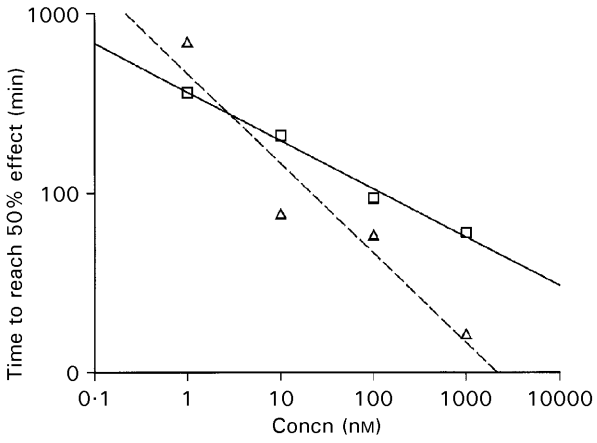

Figure 2. Correlation between the concentrations used and the time needed to induce 50% relaxation of rabbit aorta rings precontracted by KCl and incubated with lercanidipine (□) and nitrendipine (△). The r^2 values of the curves were 0·989 and 0·912, respectively, for lercanidipine and nitrendipine.

Table 1. Relaxation of rabbit aorta after incubation with different concentrations of the tested drugs.

Drug	Concentration (nM)						Slope (min nM^{-1})
	0·1	1	3	10	100	1000	
Lercanidipine	–	364 (315–413)	–	210 (203–217)	94 (67–121)	60 (59–61)	−0·270 ± 0·020
Amlodipine	–	385 (211–559)	–	278 (212–344)	133 (131–135)	57 (50–64)	−0·281 ± 0·037
Lacidipine	–	491 (174–808)	129 (112–146)	135 (120–150)	74 (60–88)	55 (52–58)	−0·269 ± 0·081
Nitrendipine	–	677 (441–913)	–	75 (62–88)	57 (48–66)	16 (nd–43)	−0·500 ± 0·110
Felodipine	426 (379–473)	327 (319–335)	60 (47–73)	70 (60–80)	–	–	−0·453 ± 0·177

Data are the times (min and 95% confidence limits) needed to induce 50% relaxation of the contraction induced by KCl, and the slope of the regression of concentration against time. nd = not determinable.

Table 2. Relaxation of rabbit aorta after incubation with different concentrations of the tested drugs.

Drug	Incubation (min)			
	60	120	180	240
Lercanidipine	1015*	46 (21–100)	13 (6·0–26)	5·6 (2·2–14)
Amlodipine	887 (316–2512)	75 (22–261)	22 (6·8–71)	9·7 (3·5–27)
Lacidipine	480 (178–1280)	17 (12–26)	2·2 (1·3–3·6)	1·7 (1·3–2·2)
Nitrendipine	53 (19–151)	7·8 (3·4–18)	4·2 (2·3–7·4)	3·1 (1·8–5·1)
Felodipine	9·1*	2·1 (1·3–3·5)	1·6 (1·2–2·0)	1·3 (1·0–1·6)

Data are the IC50 values (concentrations (nM) inducing 50% relaxation of the contraction induced by KCl), and 95% confidence limits, after different incubation times. *Extrapolated concentration (relaxation lower than 50% at all the concentrations tested).

nitrendipine and felodipine. As a consequence, the IC50 values (concentration (nM) inducing 50% relaxation of rabbit aorta) evaluated between 1 and 4 h decreased consistently with the first three drugs and markedly less after incubation with nitrendipine and felodipine (Table 2).

These last two drugs were more potent than lercanidipine, amlodipine and lacidipine after 1 h incubation, whereas after 4h incubation the potency of all the tested compounds was quite similar.

Functional Ca^{2+}-antagonistic activity on cardiac tissue (negative inotropic activity)

The addition of the vehicle used to dissolve compounds usually caused a negligible fall in heart contractility. All tested compounds induced a concentration-dependent reduction in the force of contraction (negative inotropic activity). Figure 3 shows the negative inotropic activity of lercanidipine and lacidipine as an example. Also in this model, the time needed to reach 50% reduction in the contractile force is concentration-dependent, as shown in Table 3. The ranking order of the speed in onset of the effect was lacidipine > amlodipine > felodipine > lercanidipine = nitrendipine.

The negative inotropic activity of lacidipine was not therefore clearly different when evaluated after 1 and 4 h incubation, whereas that of lercanidipine and nitrendipine was markedly higher after 4 h than after 1 h (Table 4). Nevertheless, lercanidipine had the lowest negative inotropic activity; after 4 h it was less potent than felodipine, nitrendipine, lacidipine and amlodipine by factors of 531, 426, 12·5 and 4·4, respectively.

The vasoselectivity of lercanidipine and reference compounds at different times was expressed as ratio of the IC50 values obtained for cardiac and vascular tissue; this is graphically represented in Figure 4. The vasoselectivity indices for rabbit tissues evaluated in this study proved lower than those previously published and based on the effects obtained in different tissues of different species (Guarneri et al 1996, 1997).

Nevertheless, lercanidipine was again the most vasoselective among the calcium antagonists tested at all the times considered. The IC50 ratios evaluated for lercanidipine were not substantially different at 2 and 4 h, probably because of the similarity between the rate of onset of the effects in vascular and cardiac preparations (Tables 1 and 3).

Amlodipine and, more markedly, lacidipine were more vasoselective at 3 and 4 h, whereas the vasoselectivity of nitrendipine and felodipine was lower at these times. This different behaviour might be related to the different rates of onset of the effects in the two tissues. Amlodipine and

Table 3. Negative inotropic activity on rabbit ventricle after incubation with different concentrations of the tested drugs.

Drug	Concentration (nM)								Slope1) (min nM^{-1})
	0·1	1	10	100	300	1000	10 000	30 000	
Lercanidipine	–	–	–	549 (328–770)		297 (189–763)	220 (184–256)	129 (123–135)	−0·232 ± 0·033
Amlodipine	–	–	–	366 (nd–735)		257 (182–332)	57 (54–60)	–	−0·404 ± 0·144
Lacidipine	–	–	–	551 (nd–1509)	153 (121–185)	86 (71–101)	–	–	−0·807 ± 0·177
Nitrendipine	439 (115–763)	242 (200–284)	186 (101–271)	214 (164–264)		43 (28–58)	–	–	−0·207 ± 0·066
Felodipine	–	341 (275–407)	217 (185–249)	113 (95–131)		30 (28–32)	–	–	−0·345 ± 0·062

Data are the times (min and 95% confidence limits) needed to induce 50% reduction in the basal contractile force in cardiac tissue, and the slope of the regression of concentration against time. nd = not determinable.

lacidipine, in fact, had faster effects on the heart than on the aorta whereas for nitrendipine and felodipine the onset of effect was more rapid for aorta than heart.

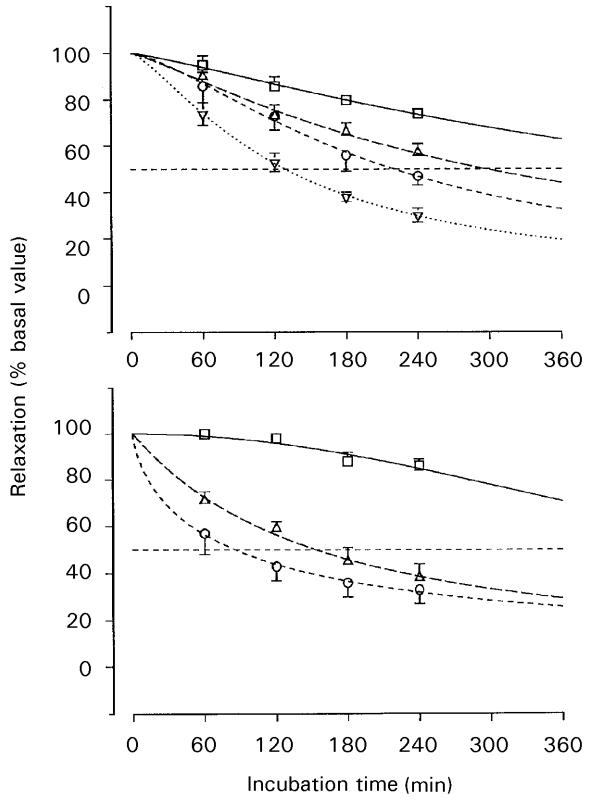

Figure 3. Negative inotropic effects induced by incubation of electrically driven rabbit heart ventricular strips with different concentrations of lercanidipine (upper) or lacidipine (lower). Lercanidipine: □ 100, △ 1000, ○ 10 000, ▽ 30 000 nM. Lacidipine: □ 100, △ 300, ○ 1000 nM.

These data also indicate that vasoselectivity should be evaluated after different incubation times. As shown in Figure 4, after 1 h incubation lacidipine was the least vasoselective compound whereas after longer incubation times nitrendipine and felodipine were the least selective of the calcium antagonists tested.

It is generally acknowledged that 1,4-dihydropyridine calcium antagonists are highly selective for vascular rather than for cardiac tissues and that this tissue selectivity distinguishes them from non-dihydropyridine calcium antagonists such as verapamil and diltiazem (Fleckenstein 1977; Opie 1988; Mecca & Love 1992). Calcium antagonists with high vascular selectivity might be preferable for the treatment of hypertension because of therapeutic advantages (Freedam and Waters 1987; Omtsuka et al 1989; Opie 1992; Packer 1992).

In this study the negative inotropic activity of lercanidipine and its vascular potency were compared with those of third-generation 1,4-dihydropyridine calcium antagonists, lacidipine and amlodipine, and with those of nitrendipine and felodipine. The vasoselectivity of these compounds is strongly related to their membrane partition coefficient in cardiac model membranes (Herbette et al 1997).

The prolonged persistence of lercanidipine in the membrane compartment might explain the long duration of its in-vivo action and the low incidence of side effects observed with this compound in clinical trials (Testa et al 1997).

In conclusion, these results confirm that lercanidipine reduces the inotropic force of the rabbit heart to a lesser extent than the other calcium

Table 4. Negative inotropic activity on rabbit ventricular strips after incubation with different concentrations of the tested drugs.

Drug	Incubation (min)			
	60	120	180	240
Lercanidipine	277 715*	37 993*	12 468 (5849–26 577)	4086 (2120–7874)
Amlodipine	8862 (3631–21 878)	2656 (1698–4074)	1554 (1202–1995)	923 (617–1412)
Lacidipine	1234*	672 (407–1122)	422 (245–741)	328 (178–617)
Nitrendipine	854 (301–2424)	151 (41–557)	25 (5·8–128)	9·6 (1·5–61)
Felodipine	302 (205–446)	69 (48–100)	16 (11–29)	7·7 (2·7–22)

Data are the IC50 values (concentrations (nM) inducing 50% reduction in the basal contractile force) and 95% confidence limits after different incubation times. *Extrapolated concentration (relaxation below 50% at all the concentrations tested).

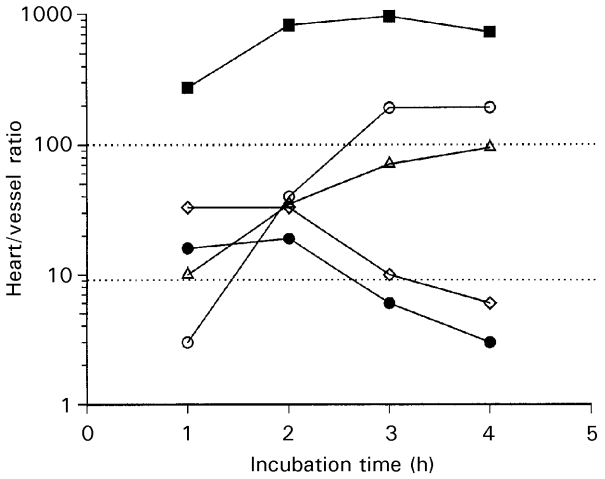

Figure 4. Vasoselectivity of lercanidipine (■), lacidipine (○), amlodipine (△), felodipine (◇), and nitrendipine (●) at different incubation times. Data represent the ratios of IC50 values for rabbit heart (concentration reducing 50% of the basal contractile force) to those for rabbit aorta (concentration inducing 50% relaxation of the precontracted tissue).

antagonists; lercanidipine also has the best heart/vessel selectivity index among the compounds tested at all the times tested.

References

Angelico, P., Guarneri, L., Leonardi, A., Testa, R. (1998) Comparative ''in-vitro'' studies on vasoselectivity of lercanidipine. J. Mol. Cell. Cardiol. 30: 762

De Lean, A., Munson, P. J., Rodbard, D. (1978) Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose–response curves. Am. J. Physiol. 235: E97–E102

Fleckenstein, A. (1977) Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. Ann. Rev. Pharmacol. Toxicol. 17: 149–166

Freedam, D., Waters, D. (1987) Second-generation dihydropyridine calcium antagonists. Drugs 34: 578–599

Guarneri, L., Angelico, P., Ibba, M., Poggesi, E., Taddei, C., Leonardi, A., Testa, R. (1996) Pharmacological in-vitro studies of the new 1,4-dihydropyridine calcium antagonist lercanidipine. Arzneim. Forsch. 46: 15–24

Guarneri, L., Sironi, G., Angelico, P., Ibba, M., Greto, L., Colombo, D., Leonardi, A., Testa, R. (1997) In-vitro and in vivo vascular selectivity of lercanidipine and its enantiomers. J. Cardiovasc. Pharmacol. 29 (Suppl. 1): S25–S32

Herbette, L. G., Rhodes, D. G., Mason, R. P. (1991) New approaches to drug design and delivery based on drug–membrane interactions. Drug Design Deliv. 7: 75–118

Herbette, L. G., Vecchiarelli, M., Leonardi, A. (1997) Lercanidipine: short plasma half-life, long duration of action. J. Cardiovasc. Pharmacol. 29 (Suppl. 1): S19–S24

Jayakody, R. L., Kappagoda, C. T., Senaratne, M. P. J. (1987) Absence of effect of calcium antagonists on endothelium-dependent relaxation in rabbit aorta. Br. J. Pharmacol. 91: 155–164

Lederballe Pedersen, O. (1981) Calcium blockade as a therapeutic principle in arterial hypertension. Clinical aspects and experimental studies on isolated vessels from spontaneously hypertensive rats and normotensive man. Acta Pharmacol. Toxicol. 49 (Suppl. 2): 31p

Leonardi, A., Motta, G., Pennini, R., Testa, R., Sironi, G., Catto, A., Cerri, A., Zappa, M., Bianchi, G., Nardi, D. (1998) Asymmetric *N*-(3,3-diphenylpropyl)aminoalkyl esters of 4-aryl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acids with antihypertensive activity. Eur. J. Med. Chem. 33: 399–420

Magnon, M., Gallix, P., Cavero, I. (1995) Intervessel (arteries and veins) and heart/vessel selectivities of therapeutically used calcium entry blockers: variable, vessel-dependent indexes. J. Pharmacol. Exp. Ther. 275: 1157–1166

Mecca, T. E., Love, S. D. (1992) Comparative cardiovascular actions of clentiazem, diltiazem, verapamil, nifedipine and nimodipine in isolated rabbit tissues. J. Cardiovasc. Pharmacol. 20: 678–682

Omtsuka, M., Yokota, M., Kodama, I., Yamada, K. (1989) New generation dihydropyridine calcium entry blockers in search of greater selectivity for one tissue subtype. Gen. Pharmacol. 20: 539–556

Opie, L. H. (1988) Calcium channel antagonists. Cardiovasc. Drugs Ther. 1: 411–430

Opie, L. H. (1992) Should calcium antagonists be used after myocardial infarction? Ischemia selectivity versus vascular selectivity. Cardiovasc. Drugs Ther. 6: 19–24

Packer, M. (1992) Second generation calcium-channel blockers in the treatment of chronic heart failure: are they any better than their predecessors? Am. J. Cardiol. 14: 1339–1341

Testa, R., Leonardi, A., Tajana, A., Riscassi, E., Magliocca, R., Sartani, A. (1997) Lercanidipine (Rec 15/2375): a novel 1,4-dihydropyridine calcium antagonist for hypertension. Cardiovasc. Drug. Res. 15: 187–219