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Dual Mode of Action of Dihydropyridine Calcium Antagonists

A Role for Nitric Oxide

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Contents

Abstract

Dihydropyridine calcium antagonists have been used for many years in the treatment of angina pectoris and hypertension. According to the common view, their mechanism of action is based on an inhibition of the smooth muscle L-type calcium current, thus decreasing intracellular calcium concentration and inducing smooth muscular relaxation. However, in recent years evidence has accumulated that besides the smooth muscle effects of these agents, their effect on the endothelium must also to be taken into account.

It was shown that dihydropyridines can induce the release of nitric oxide (NO) from the vascular endothelium of various vessels and in different species. This was first shown by Günther and colleagues by assaying the methaemoglobin formation in the presence of intact endothelium (in procine coronary arteries) with and without treatment with nitrendipine. These findings were later confirmed by direct measurement of NO or of nitrite production. In addition, in several preparations, including micro- and macrovasculature, the sensitivity of the vasorelaxing effect of the dihydropyridines to inhibitors of NO-synthase, such as L-N^G-nitroarginine (LNNA) or L-N-nitro-arginine-methyl-ester (L-NAME), has been shown. With these studies it became evident that the NO-releasing effect was not unique to nitrendipine but was a group phenomenon shared by the dihydropyridines and several nondihydropyridine calcium antagonists. In addi-

tion to their action on vascular endothelium, NO release by nifedipine has also been detected in platelets. There are also studies showing long term effects of calcium antagonists involving NO release.

Regarding the underlying mechanism of NO release, nitrendipine was shown, not to decrease but to increase intracellular Ca^{2+} in cultured endothelial cells. This increase was sensitive to both Ca^{2+}-free extracellular superfusion and to gadolinium, a lanthanide known to inhibit shear-stress activated cation channels. This increase in intracellular calcium can activate endothelial NO-synthase, thus inducing the release of NO.

These findings on a dual mode of action, i.e. the direct relaxing effect by inhibition of the smooth muscle L-type calcium current and indirect relaxing effect by release of NO from vascular endothelium may help to understand the beneficial antihypertensive effects of the dihydropyridine calcium antagonists and the preferential effect of certain drugs in certain vascular regions (resistance versus conductive vessels). In addition, NO release from both vascular endothelium and platelets may contribute to the antiatherosclerotic and antithrombotic effects described for certain dihydropyridines.

Calcium antagonists are widely used drugs in cardiovascular medicine for the treatment of cardiac arrhythmias and angina pectoris,and as antihypertensive agents.[1] The group of calcium antagonists includes 5 classes of different chemical structure: phenylalkylamines, benzothiazepines, diphenyl-piperazines, diarylaminopropylamines and 1,4-dihydropyridines.[1] Among the antihypertensive agents, the dihydropyridines are very commonly used. The mode of action of these drugs is theoretically based on the inhibition of calcium channels in the smooth muscle cells of the vascular wall.[1] The resulting decrease in intracellular Ca^{2+} ($[Ca^{2+}]_i$] finally causes smooth muscle relaxation.

There are at least 3 different mechanisms responsible for smooth muscular contraction: (i) influx of Ca^{2+} via voltage sensitive calcium channels in response to membrane depolarisation; (ii) agonist-induced release of Ca^{2+} from sarcoplasmic reticulum via hydrolysis of membrane phosphatidylinositol (the released Ca^{2+} may trigger further influx of extracellular calcium); and (iii) influx of Ca^{2+} via receptor-operated calcium channels. The voltage-dependent calcium channels can be divided into 3 subgroups with regard to their electrophysiological behaviour: L-, T- and N-type channels.[2,3] Calcium antagonists inhibit voltage-dependent calcium channels in the commonly used therapeutic concentration range. The dihydropyridines affect predominantly L-type calcium channels. Commonly, calcium antagonists relax arterial vessels and have little effect on venous vascular beds.

Since the discovery of endothelium-dependent vasorelaxation by Furchgott and Zawadzki in 1980,[4] scientific interest has been attracted to the role of the endothelium in vascular regulation. After the identification of the endothelium-derived relaxing factor (EDRF) as nitric oxide (NO) by Ignarro et al.[5] and Palmer et al.,[6] the relaxant action of nitroglycerin and sodium nitroprusside could be attributed to the release of NO. An important question in that context was whether endothelium-derived NO may be involved in other drug-induced vasorelaxation.

1. Vasorelaxation by Dihydropyridines: A Role for Nitric Oxide (NO)

1.1 Early Experiments with Nitrendipine

Experiments were performed to evaluate the role of the endothelium in vasorelaxation induced by dihydropyridines. NO activates soluble guanylate cyclase[7] and methylene blue is known to block guanylate cyclase, therefore, this substance was used to inhibit endothelium-dependent relaxation. Early experiments using methylene blue in prosta-

glandin (PG)$F_{2\alpha}$-preconstricted coronary arteries, showed a reduced response for nitrendipine.[8] In subsequent studies,[9] the authors found similar results in porcine basilar arteries with various dihydropyridines. In the context of these studies, dihydropyridines were shown to be less effective in endothelium-denuded vessels than in intact vessels. Although this could also be attributable to the preparation procedure and to a possible damage of the smooth muscle layer during denudation, these experiments (taken together) gave the first hint of a possible involvement of endothelium in dihydropyridine-induced vasodilation.

These experiments suggested a possible role of endothelium derived mediators in dihydropyridine-induced vasorelaxation. Therefore, the effect of nitrendipine (as a typical dihydropyridine) on porcine coronary, basilary and tail arteries in the absence and presence of L-N^G-nitroarginine (LNNA), a potent inhibitor of constitutive NO-synthase,[10] was investigated.[11] There was a reduced response to nitrendipine if endothelial NO-synthase (eNOS) was blocked by LNNA. Consequently, the same investigators examined NO release from intact porcine coronary arteries under the influence of nitrendipine using the methaemoglobin method.[12] The formation of methaemoglobin was increased following the administration of nitrendipine (0.1 to 10 μmol/L) in these experiments indicating that nitrendipine induced NO-release from the endothelium.[11] The increased formation of methaemoglobin could be prevented by simultaneous application of LNNA 3 μmol/L. This was the first direct evidence for dihydropyridine-induced NO release from vascular endothelium.

1.2 Effect of Drug and Vessel

The next question was whether this action is restricted to nitrendipine only or whether other dihydropyridines may also release NO. In addition, it remained to be elucidated whether such a possible drug-induced NO release may be different in various vessels. Therefore, common dihydropyridines such as nifedipine, nisoldipine, nimodipine, and for comparison nitrendipine, were investigated in the guinea pig mesenteric arterial network.[13,14] For that purpose, mesenteric arterial networks were isolated, perfused at constant pressure and observed using microvideoangiometry. Conductive and resistive vessels were investigated, i.e. arteries of 600, 400, 300 and 170μm diameter. The various dihydropyridines were infused in increasing concentrations with and without application of the eNOS inhibitor LNNA. The vasodilating and flow-increasing effects of all 4 drugs could be significantly reduced by blocking eNOS.

Interestingly, the highest effect of eNOS inhibition was seen in small arteries (<300μm) relaxed by nifedipine or nitrendipine. Because in the cumulative concentration-response curves the E_{max} value for vasorelaxation was significantly reduced by additional LNNA, while the EC_{50} was not affected, the authors assumed a noncompetitive interaction between both substances, i.e. between the dihydropyridines and LNNA. It was concluded that NO release significantly contributes to vasorelaxation induced by nifedipine or nitrendipine 1 to 10 nmol/L in this model and, thus, this may occur in therapeutically used concentrations; that NO-release from the endothelium seems to be a group phenomenon of dihydropyridines, although the extent of this action may differ between the various agents; and, finally, that the NO-releasing effect may be of greater importance in small arteries.[14]

After evidence for NO release by dihydropyridines had accumulated using the methaemoglobin method and functional studies with eNOS inhibition with LNNA, further experiments were carried out using direct measurement of NO. Utilising an NO-sensitive technique, a direct demonstration of NO release from the endothelium was shown using therapeutic concentrations of nifedipine in porcine coronary arteries.[15]

1.3 Proposed Mechanism of Action

As the next step in the investigation of a putative role for NO in dihydropyridine-induced vasorelaxation, studies were undertaken to elucidate the underlying mechanism of action. At first

glance, the finding of enhanced NO release under the influence of a calcium antagonist seemed astonishing, given that eNOS is a Ca^{2+}-dependent enzyme, especially as other investigators found a reduction in stimulated EDRF-induced relaxation in the presence of calcium antagonists.[16] However, these investigators did not examine the influence of dihydropyridines on the spontaneous release of NO but on stimulated NO-dependent relaxation. On the other hand, it is known that macrovascular endothelial cells do not express L-type calcium channels and, thus, the typical target for dihydropyridine binding is lacking on these cells. Although, in microvascular endothelial cells, a new type of Bay K 8644-sensitive channel has been characterised.[17] However, a nonselective cation channel has also been described on endothelial cells, which exhibits some preference for Ca^{2+}.[18] Thus, the question arose as to whether dihydropyridines affect $[Ca^{2+}]_i$ concentrations in endothelial cells and, if so, by which mechanism.

In order to shed light on this problem, Salameh and co-workers[19] investigated the effect of nitrendipine on suspended cultured bovine aortic endothelial cells using the fura-2 technique. They found a significant increase in intracellular $[Ca^{2+}]_i$ following the administration of nitrendipine 0.1, 1 and 10 μmol/L. The signal did not exhibit a peak and plateau phase as can be observed after bradykinin but was steadily increasing reaching steady state conditions after 3 minutes. This elevation in $[Ca^{2+}]_i$ could be completely suppressed by the application of gadolinium, a trivalent lanthanide known to inhibit shear stress-activated cation channels on endothelial cells,[20,21] and by incubation in Ca^{2+} free saline solution, indicating that the dihydropyridine-induced elevation of $[Ca^{2+}]_i$ is mainly due to a gadolinium-sensitive influx of Ca^{2+} from the extracellular space.

Additional experiments showed that the nitrendipine-induced NO release was also sensitive to gadolinium treatment.[19] The lack of effect of thapsigargin pretreatment, which leads to depletion of intracellular calcium stores, indicates that intracellular release of Ca^{2+} was not predominantly involved. Because the gadolinium sensitivity pointed to a possible involvement of shear stress-activated Ca^{2+} influx, the effect of nitrendipine at various shear stresses was investigated. Suspensions of fura-2 loaded endothelial cells were stirred at various speeds and the fura-2 fluorescence indicating the $[Ca^{2+}]$ was monitored. A shear stress-dependent increase in $[Ca^{2+}]_i$ could be observed which was significantly enhanced in the presence of nitrendipine.[19] Because of the sensitivity to gadolinium and since endothelial cells from macrovascular arteries do not express L-type channels,[22,17] Salameh and colleagues[19] concluded that the nitrendipine-induced elevation in $[Ca^{2+}]_i$ is not mediated via interaction with L-type calcium channels but may involve Ca^{2+}-influx via shear stress activated cation selective channels.

1.4 Various Calcium Antagonists

After these observations, several other studies were performed to investigate a possible involvement of NO in calcium antagonist-induced vasorelaxation. In these studies,[23-26] it was demonstrated that the vasorelaxation induced by other dihydropyridines also involves endothelial release of NO. This was shown for isradipine and lacidipine in human middle cerebral artery,[23] while nifedipine exhibited only minor endothelium dependence in that vascular region. Pranidipine, a new dihydropyridine, was recently shown to prolong acetylcholine- or nitroglycerin-induced vasorelaxation via a LNNA-dependent mechanism.[26] However, since the effect was insensitive to methylene blue, it probably did not involve soluble guanylate cyclase and, thus, may differ from that of the other dihydropyridines.

Recently, NO release was also confirmed for amlodipine (1 nmol/L to 10 μmol/L) in large canine coronary arteries, canine coronary microvessels and canine aorta measuring nitrite production and sensitivity to L-N-nitro-arginine-methyl-ester (L-NAME).[27] The effect of the drug on each vessel was similar, although a direct comparison between vessels is difficult in this study because 20mg of tissue from each vessel type was investigated, so

that the intimal surface should be different as a result of the differences in diameter and wall thickness. In these canine vessels, nifedipine did not cause NO release. The amlodipine-induced NO release could also be suppressed with Hoe140 and dichloroisocoumarin, indicating that the signal transduction may involve kinin-2 receptors and kinin formation as well. However, only a single dose was studied so that it is not possible to conclude from the study whether the interaction between amlodipine and Hoe140 was competitive.

An enhancement of $[Ca^{2+}]_i$ in cultured bovine endothelial cells by the non-dihydropyridine calcium antagonists dotarizine and diltiazem has also been shown,[24] although the effect of these drugs was considerably lower than that of nitrendipine. Furthermore, a sensitivity to NO-synthase inhibition was shown in coronary arteries relaxed by the benzothiazepine calcium antagonist clentiazem in Syrian hamsters.[25]

Finally, an enhanced release of EDRF has also described for fendiline and calmidazolium.[28]

1.5 Long Term Use

The long term effects of these drugs on endothelial and vessel function are of special interest because calcium antagonists are often used for the long term treatment of hypertension. Therefore, several studies have been performed to investigate the involvement of NO in the long term effects of calcium antagonists. Endothelial dysfunction, as characterised by reduced dilatory response to acetylcholine in the mesenteric resistance arteries, occurs within 21 weeks of age in spontaneously hypertensive rats (SHR). This dysfunction could be prevented with an ACE-inhibitor but not with the dihydropyridine amlodipine.[29] In contrast, in normal Wistar-Kyoto rats receiving the NO-synthase inhibitor L-NAME, the resulting blunted dilatory response to acetylcholine could be prevented by long term (6 weeks) treatment with verapamil.[30] In renal resistance arteries of SHR, however, the reduced dilatory response to acetylcholine could be improved by administration of benidipine for 10 weeks.[31] Furthermore, the hyperresponsiveness of mesenteric vasculature to phenylephrine induced by L-NAME treatment in normal rats could be inhibited by diltiazem.[32] Thus, there are hints on possible long term effects of antihypertensive treatment with calcium antagonists which involves their effects on NO release.

1.6 NO Release from Platelets

The NO-releasing action of nifedipine has also been observed in platelets.[33] Porcine platelets were stimulated with collagen in the absence or presence of nifedipine. Nifedipine inhibited collagen-induced platelet aggregation with an IC_{50} of 380 nmol/L. This antiaggregatory action was suppressed by LNNA. Direct measurement of NO with a NO-sensitive electrode confirmed nifedipine-induced NO release from porcine platelets.

1.7 Unanswered Questions

In summary, the release of NO from endothelial cells (i) seems to be a group phenomenon of, at least, the dihydropyridines (and possibly of some other calcium antagonists), which seems to depend to different degrees on vessel type, drug and species; (ii) involves increases in endothelial $[Ca^{2+}]_i$ and activation of eNOS; (iii) the signal transduction may involve Ca^{2+}-influx via shear stress activated cation channels or other mechanisms as, for example, tyrosine kinase activation or kinin receptors and kinin formation. The phenomenon is not restricted to endothelial cells alone and also occurs in platelets.

Currently unanswered questions are: (i) what is the exact molecular mechanism of action; (ii) what is the underlying principle for the differences between the drugs with regard to the extent of the effect and to the preferred vascular region; and (iii) what are the clinical consequences, especially in long term treatment?

1.8 Alternative Hypothesis

Finally, although evidence has accumulated that NO is involved in the vasorelaxing effect of the dihydropyridines,[8-11,13-15,19,23-24,27] there are some

alternative hypotheses to explain the endothelial-related vasorelaxing effect of dihydropyridines that are worth discussing. One could suppose synergistic effects with other vasodilators such as prostacyclin (enhanced release of prostacyclin and modulation of cholesterol metabolism has been demonstrated earlier for several calcium antagonists including dihydropyridines),[34-36] an interaction via scavenging of free radicals[37-39] which otherwise might interfere with NO, or antagonism of an endogenously released vasoconstrictor such as angiotensin II, $PGF_{2\alpha}$ or endothelin. Some calcium antagonists may exert an inhibitory effect on noradrenaline (norepinephrine) release from sympathetic perivascular nerve terminals.[40] Complex interactions between the drug administered and the broad variety of vasoactive mediators present in blood and vascular wall may occur in the vasculature finally leading to an enhanced release of NO or any other contribution of the endothelium to the vasorelaxant effect. However, besides the studies mentioned above, there is evidence for a direct effect of dihydropyridines on endothelial Ca^{2+} homeostasis and NO-release, which was shown in cultured endothelial cells.[19]

2. Conclusions and Possible Clinical Implications

The present data shows a dual mode of action for calcium antagonists: the well known relaxation of arteries via inhibition of voltage-operated L-type calcium channels and, secondly, a release of NO from endothelium and possibly from platelets. This action is shared by many calcium antagonists, although to a variable extent, and seems to resemble a group phenomenon. A schematic survey of the pharmacodynamics is given in figure 1.

What is the clinical relevance of these findings? First of all, a contribution of NO release to the vasodilation induced by calcium antagonists may help to explain the vascular selectivity of some of the drugs and may help in understanding the

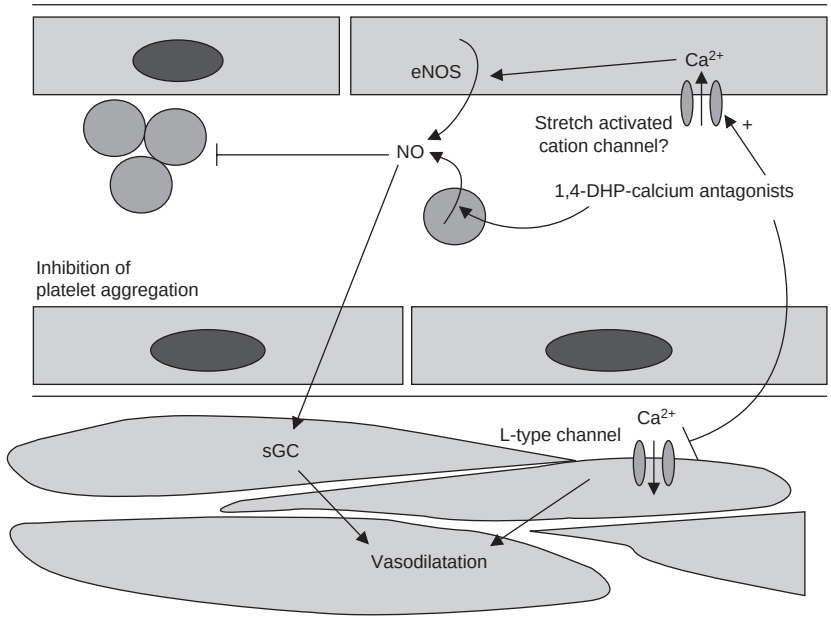

Fig. 1. A schematic survey of the pharmacodynamics of the dual mode of action of dihydropyridine (DHP) calcium antagonists: (1) relaxation of arteries via inhibition of voltage-operated L-type calcium channels; and (2) release of nitric oxide (NO) from the endothelium and possibly from platelets. **eNOS** = endothelial NO synthase; **sGC** = soluble guanylate cyclase.

preferential effect on certain vascular regions or branches (e.g. resistance versus conductive vessels) that was previously suggested.[14] Clinically, an antiatherosclerotic action of nifedipine characterised by a retardation of the angiographic progression of coronary artery disease has been observed.[41] Experimentally, vasoprotective and antiproliferative effects of calcium antagonists have also been shown.[42] These antiatherosclerotic, antithrombotic and antiproliferative actions could be at least partially influenced by an additional release of NO from the endothelium and platelets. More studies, especially on long term effects of treatment with calcium antagonists on vascular performance and endothelial function in pathophysiological models or in the clinical situation, would be highly interesting. The dual mode of action of calcium antagonists, inhibition of L-type calcium channels and release of NO, may help to improve our understanding of the action of these drugs.

References

1. Fleckenstein A. Specific pharmacology of calcium in myocardium, cardiac pacemakers and vascular smooth muscle. Ann Rev Pharmacol Toxicol 1977; 17: 149-66
2. Tsien RW, Lipscombe D, Madison DV, et al. Multiple types of neuronal calcium channels and their selective modulation. Trends Neurosci 1988; 11: 431-8
3. Schwartz A. Molecular and cellular aspects of calcium channel antagonism. Am J Cardiol 1992; 70: 6-8F
4. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-9
5. Ignarro L, Byrus RE, Buga GM, et al. Endothelium-derived relaxing factor from pulmonary artery and vein possesses pharmacologic and chemical properties identical to those of nitric oxide. Circ Res 1987; 61: 866-79
6. Palmer RMJ, Ferridge AG, Moncada S. Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524-6
7. Förstermann U, Mülsch A, Böhme E, et al. Stimulation of soluble guanylate cyclase by an acetylcholine-induced endothelium-derived relaxing factor from rabbit and canine arteries. Circ Res 1986; 58: 531-8
8. Kojda G, Werner G, Klaus W, et al. Reduced responses of nitrendipine in $PGF_{2\alpha}$ precontracted porcine isolated arteries after pretreatment with methylene blue. Basic Res Cardiol 1990; 85: 461-6
9. Kojda G, Klaus W, Werner G, et al. The influence of endothelium on the action of $PGF_{2\alpha}$ and some dihydropyridine type calcium antagonists in porcine basilar arteries. Basic Res Cardiol 1991; 86: 254-65
10. Ishii K, Chang B, Kerwin JF, et al. N-Nitro-L-Arginine: a potent inhibitor of endothelium-derived relaxing factor formation. Eur J Pharmacol 1990; 176: 219-23
11. Günther J, Dhein S, Rösen R, et al. Nitric oxide (EDRF) enhances the vasorelaxing effect of nitrendipine in various isolated arteries. Basic Res Cardiol 1992; 87: 452-60
12. Feelisch M, Noack EA. Correlation between NO formation during degradation of organic nitrates and activation of guanylate cyclase. Eur J Pharmacol 1987; 139: 19-30
13. Dhein S, Zhao Y, Klaus W. Microvascular actions of dihydropyridines in isolated mesenteric vascular beds and the involvement of nitric oxide [abstract]. Proceedings of the IBCs 3rd Symposium on Nitric Oxide; 1994 Mar 17-18; Philadelphia (PA): Abstract Book 1994; PB#22
14. Dhein S, Zhao Y, Simsek S, et al. Actions of dihydropyridines in isolated mesenteric vascular beds. J Cardiovasc Pharmacol 1995; 26: 784-91
15. Berkels R, Bertsch A, Breitenbach T, et al. The calcium-antagonist nifedipine stimulates endothelial NO release in therapeutical concentrations. Pharmaceut Pharmacol Lett 1996; 6: 75-8
16. Rubanyi G, Schwarz A, Vanhoutte PM. Calcium transport mechanisms in endothelial cells regulating the synthesis and release of endothelium-derived relaxing factor. In: Vanhoutte PM, editor. Relaxing and contracting factors. Crescent Manor: Humana Press, 1988: 179-88
17. Bossu JL, Elhamdani A, Feltz A, et al. Voltage gated Ca entry in isolated bovine capillary endothelial cells: evidence for a new type of Bay K 8644 sensitive channel. Pflügers Arch 1992; 402: 200-7
18. Nilius B, Riemann D. Ion channels in human endothelial cells. Gen Physiol Biophys 1990; 9: 89-112
19. Salameh A, Schomecker G, Breitkopf K, et al. The effect of the calcium-antagonist nitrendipine on intracellular calcium concentration in endothelial cells. Br J Pharmacol 1996; 118: 1899-904
20. Naruse K, Sokabe M. Involvement of stretch-activated ion channels in Ca^{++}-mobilization to mechanical stretch in endothelial cells. Am J Physiol 1993; 264: C1037-44
21. Hishikawa K, Nakai T, Marumo T, et al. Pressure enhances endothelin-1 release from cultured human endothelial cells. Hypertension 1995; 25: 449-52
22. Colden-Stanfield M, Schilling WP, Ritchie AK, et al. Bradykinin-induced increases in cytosolic calcium and ionic currents in cultured bovine aortic endothelial cells. Circ Res 1987; 61: 632-40
23. Akopov SE. Nitric oxide (EDRF) enhances the vasorelaxing effect of dihydropyridine calcium antagonists in isolated human middle cerebral arteries. Methods Find Exp Clin Pharmacol 1996; 18: 101-4
24. Salameh A, Eisert A, Schomecker G, et al. Effects of nitrendipine, dotarizine and diltiazem on intracellular calcium in cultured bovine aortic endothelial cells [abstract]. Pharmacol Res 1995; 31 Suppl.: 227
25. Tanguay M, Jasmin G, Baise G, et al. Coronary and cardiac sensitivity to the vasoselective benzothiazepine-like calcium antagonist, centiazem, in experimental heart failure. Cardiovasc Drugs Ther 1997; 11: 71-9
26. Mori T, Takeuchi T, Ohura M, et al. Pranidipine, a new dihydropyridine calcium channel blocker, enhances cyclic GMP-independent nitric oxide-induced relaxation of the rat aorta. Mol Cell Biochem 1998; 178: 335-43
27. Zhang X, Hintze TH. Amlodipine releases nitric oxide from canine coronary microvessels: an unexpected mechanism of action of a calcium channel-blocking agent. Circulation 1998; 97: 576-80

28. Busse R, Lückhoff A, Winter I, et al. Fendiline and calmidazolium enhance the release of endothelium-derived relaxing factor and of prostacyclin from cultured endothelial cells. Naunyn Schmiedeberg's Arch Pharmacol 1988; 337: 79-84
29. Bennett MA, Hillier C, Thurston H. Endothelium-dependent relaxation in resistance arteries from spontaneously hypertensive rats: effect of long-term treatment with perindopril, quinapril, hydralazine or amlodipine. J Hypertens 1996; 14: 389-97
30. Takase H, Moreau P, Kung CF, et al. Antihypertensive therapy prevents endothelial dysfunction in chronic nitric oxide deficiency: effect of verapamil and trandolapril. Hypertension 1996; 27: 25-31
31. Dohi Y, Kojima M, Sato K. Benidipine improves endothelial function in renal resistance arteries of hypertensive rats. Hypertension 1996; 28: 58-63
32. Navarro-Cid J, Maeso R, Rodrigo E, et al. Renal and vascular consequences of the chronic nitric oxide synthase inhibition: effects of antihypertensive drugs. Am J Hypertens 1996; 9: 1077-83
33. Berkels R, Klaus W, Boller M, et al. The calcium modulator nifedipine exerts antiaggregatory property via a nitric oxide mediated process. Thrombosis Haemostasis 1994; 72: 309-12
34. Kirschenbaum MA, Roh DD, Kamanna VS. Effect of nifedipine on renal microvascular cholesterol accumulation and prostacyclin biosynthesis in cholesterol-fed rabbits. Atherosclerosis 1991; 91: 241-6
35. Nicholson AC, Etingin OR, Pomerantz KB, et al. Dihydropyridine calcium antagonist modulates cholesterol metabolism and eicosanoid biosynthesis in vascular cells. J Cell Biochem 1992; 48: 393-400
36. Pirich C, Schmid P, Fitscha P, et al. Influence of calcium antagonists on platelet function and vascular prostacyclin production. Blood Press 1994; Suppl. 1: 75-80
37. Van Amsterdam FT, Roveri A, Maiorino M, et al. Lacidipine: a dihydropyridine calcium antagonist with antioxidant activity. Free Radic Biol Med 1992; 12: 183-7
38. Jacinto SM, Jandhyala BS. Evaluation of the effects of felodipine, verapamil and hydralazine on the survival rate of rats subjected to lethal effects of oxygen free radicals. Naunyn Schmiedeberg's Arch Pharmacol 1992; 346: 457-61
39. Reddy DS, Singh M, Chopra K. Comparative antioxidant effects of beta-adrenoceptor blockers, calcium antagonists and U74500A against iron-dependent lipid peroxidation in murine ventricular microsomal membranes. Methods Find Exp Clin Pharmacol 1996; 18: 559-67
40. Tsuda K, Kuchii M, Nishio I, et al. Effects of calcium-antagonists on norepinephrine release from sympathetic nerve endings in rat mesenteric vasculature. Jpn Heart J 1986; 27: 395-402
41. Lichtlen PR, Hugenholz PG, Rafflenbeul W. Retardation of angiographic progression of coronary artery disease by nifedipine. Lancet 1990; 335: 1109-13
42. Fleckenstein-Grün G, Thimm F, Czirfuzs A, et al. Experimental vasoprotection by calcium antagonists against calcium-mediated arteriosclerotic alterations. J Cardiovasc Pharmacol 1994; 24 Suppl. 2: 75-84

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