Novel Vascular Biology of Third-Generation L-Type Calcium Channel Antagonists
Ancillary Actions of Amlodipine

R. P. Mason, P. Marche, T. H. Hintze

Abstract—Calcium channel blockers (CCBs) were developed as vasodilators, and their use in cardiovascular disease treatment remains largely based on that mechanism of action. More recently, with the evolution of second- and third-generation CCBs, pleiotropic effects have been observed, and at least some of CCBs’ benefit is attributable to these mechanisms. Understanding these effects has contributed greatly to elucidating disease mechanisms and the rationale for CCB use. Furthermore, this knowledge might clarify why drugs are useful in some disease states, such as atherosclerosis, but not in others, such as heart failure. Although numerous drugs used in the treatment of vascular disease, including statins and angiotensin-converting–enzyme inhibitors, have well-described pleiotropic effects universally accepted to contribute to their benefit, little attention has been paid to CCBs’ potentially similar effects. Accumulating evidence that at least 1 CCB, amlodipine, has pharmacologic actions distinct from L-type calcium channel blockade prompted us to investigate the pleiotropic actions of amlodipine and CCBs in general. There are several areas of research; foci here are (1) the physicochemical properties of amlodipine and its interaction with cholesterol and oxidants; (2) the mechanism by which amlodipine regulates NO production and implications; and (3) amlodipine’s role in controlling smooth muscle cell proliferation and matrix formation. (Arterioscler Thromb Vasc Biol. 2003;23:2155-2163.)

Key Words: membrane fluidity ■ superoxide ■ nitric oxide ■ cholesterol ■ smooth muscle proliferation

A number of studies have suggested that vascular injury and cardiovascular disease (CVD) associated with hypertension, including atherosclerosis, manifesting itself as stroke and myocardial infarction, can be modified by reducing arterial pressure. Whether it is the mechanism of reducing blood pressure (BP) or the reduction in BP per se that is most important is a matter of some debate. For instance, the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) has recently shown that a vasodilator such as amlodipine is as effective as a diuretic in reducing cardiovascular complications. Thus, if BP reduction is a goal, then understanding the mechanisms by which drugs reduce BP and consequently tailoring therapies based on insight into underlying disease mechanisms become other significant goals.

A number of drugs currently used in the treatment of CVD, including hypertension and atherosclerosis, have been shown to have significant pleiotropic actions. These drugs include angiotensin-converting–enzyme (ACE) inhibitors, which potentiate kinin–nitric oxide (NO) production; statins, which not only lower plasma cholesterol but also scavenge superoxides and promote NO biosynthesis; and calcium channel blockers (CCBs), which have multiple actions and are the focus of this review. Although the clinical actions of these drugs are still largely attributed to their primary known mechanisms of action, it is likely that pleiotropic actions contribute significantly to the benefits they produce. The lack of concrete evidence demonstrating the value of these pleiotropic actions in the clinical setting might stem from use of incorrect dosages, lack of penetration of the drug to the site where the pleiotropic action is initiated, or interactions between pleiotropic effects and the primary action of the drugs. Thus, pleiotropic effects might make drugs more useful; alternatively, they might explain the limitations to drug benefit in some diseases, such as heart failure and hypertension.

The first-generation dihydropyridine L-type CCBs, such as nifedipine, were developed as selective and powerful vasodilators, partly in an effort to avoid the heart rhythm disturbances and perhaps the negative inotropic effects caused by calcium channel blockade in the heart that had limited the use of the phenylalkylamine-type CCB verapamil. To this end, nifedipine has proved to be very successful and...
is widely used in the treatment of vascular diseases, including hypertension and angina. Furthermore, as a result of CCBs’ effective control of afterload (arterial pressure) and the potential value of an increase in coronary blood flow for the treatment of both cardiac ischemia and heart failure, they have had much wider clinical application.

To increase the selectivity of nifedipine, a series of second-generation CCBs, eg, nimodipine, was developed by altering nifedipine’s physicochemical and pharmacodynamic properties. In addition, concerns centering on the short half-life of nifedipine led to the development of a long-acting formulation, nifedipine gastrointestinal therapeutic system (GITS), the agent used in the International Nifedipine-GITS Study: Intervention as a Goal in Hypertension Treatment (INSIGHT). A third-generation series of L-type CCBs has also been developed, and at least 1 of these, amlodipine, has shown additional promise in the treatment of both vascular and vessel disease. In ALLHAT, amlodipine was evaluated against a diuretic in the treatment of hypertension and was found to be as useful in the control of arterial pressure. A survey of a number of clinical trials with CCBs suggested that their ability to control arterial pressure was of paramount importance. In the Circadian Anti-ischemia Program in Europe (CAPE) trial, amlodipine was shown to reduce ischemia in patients with coronary artery disease; in the Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT), amlodipine was shown to reduce hospitalizations for unstable angina and revascularization; and in the Coronary Angioplasty Amlodipine RESTenosis Study (CAPARES), amlodipine was shown to reduce the need for revascularization in patients with stable angina.

As recently reviewed in this Journal, the pleiotropic effects of statins might serve to explain some of their unexpected mechanisms and actions, both in vitro and in clinical settings. The same holds true for CCBs. In this respect, elucidation of the ancillary actions of amlodipine might lead to a better understanding of the (1) biochemical and physiologic actions of the drug, (2) potential use of the drug in the treatment of vascular and cardiac diseases, (3) limitations of the drug and its apparent selectivity for certain CVDs, and (4) potential interaction of the drug with other drugs also used in treating CVD. In addition, insights gained from an understanding of the mechanisms of action of amlodipine might help to clarify the mechanistic contribution of related drugs; eg, another CCB, benidipine, appears to have effects similar to those of amlodipine. Thus, the goal of this review is to highlight the pleiotropic actions of amlodipine, the relevance of these actions in similar drugs, and their role in therapeutics. The role of L-type calcium channel blockade as a mechanism leading to vasodilation (Figure 1) will not be discussed unless germane to a pleiotropic action.

Physicochemical Properties Specific to Certain CCBs That Might Enable Vascular Effects

Beyond causing vasodilation through inhibition of calcium channels, long-acting CCBs have been demonstrated to produce clinical benefits in patients with coronary artery disease that might be independent of BP changes. The basis for these additional vascular effects might be related to the physicochemical properties of these agents. In the case of amlodipine, for instance, >90% of the aminoethoxy function associated with the dihydropyridine ring of amlodipine is in the charged state at physiologic pH levels. This positive charge leads to strong electrostatic interactions of amlodipine with membrane phospholipid head groups. These physicochemical interactions result in the drug’s concentrating in the membrane at levels >10 000-fold higher than in the surrounding aqueous environment. At these high membrane concentrations, there is a sustained reservoir of amlodipine available for binding over time to calcium channel receptors within the plasma membrane of vascular smooth muscle cells (VSMCs). These measurements explain the extended duration of activity of amlodipine compared with shorter-acting, less lipophilic agents. The high membrane affinity of amlodipine compared with that of other CCBs was preserved even under atherosclerosis-like conditions characterized by an increase in membrane cholesterol content. Additionally, the positive charge and strong lipid affinity of amlodipine enable it to inhibit aggregation of modified LDLs, a key step in foam cell formation mediated by the electronegative properties of oxidized lipid. This atheroprotective effect of amlodipine could not be reproduced by other antihypertensive agents (other CCBs, ACE inhibitors) that lack such physicochemical characteristics.

The precise membrane location of amlodipine has been determined by biophysical approaches, including small-angle x-ray diffraction, differential scanning calorimetry, and proton nuclear magnetic resonance. The dihydropyridine ring structure of amlodipine resides at the same depth as the sterol nucleus of cholesterol; at this location, the drug can
reverse the adverse effects of cholesterol on membrane structure and function.\textsuperscript{13,19–21} In particular, amlodipine can interfere with the ability of cholesterol to increase membrane width\textsuperscript{20,21} and to aggregate into discrete, crystalline-like domains, as has been described for both native and oxidized derivatives of cholesterol in models of atherosclerotic disease.\textsuperscript{22,23}

Strong membrane interactions have been reported for other third-generation CCBs, such as lacidipine and lercanidipine.\textsuperscript{24,25} In the case of lacidipine, the molecular structure favors hydrophobic interactions with membrane phospholipid acyl chains. Consistent with this chemical structure, x-ray diffraction studies indicate that the molecule is located deep in the membrane hydrocarbon core and concentrates in the membrane at a very high level at equilibrium.\textsuperscript{24,25} The high affinity of lacidipine for vascular membranes under normal and atherosclerosis-like conditions might contribute to the antiatherogenic activities reported for this compound in preclinical models of atherosclerotic disease.\textsuperscript{26,27}

**Antioxidant Properties of CCBs**

A pleiotropic effect reported for CCBs that might affect the development of atherosclerosis is the ability of these agents to reduce oxidative modification of LDLs and membrane lipids. Oxidative modification of LDL and membrane lipids contributes to foam cell formation, endothelial dysfunction, and destructive inflammatory processes associated with atherosclerosis.\textsuperscript{28,29}

Both in vitro and in vivo studies have shown that highly lipophilic CCBs inhibit oxidative damage to lipids associated with cellular membranes and lipoprotein particles.\textsuperscript{30,31} Under controlled experimental conditions, amlodipine inhibited lipid peroxide formation at concentrations as low as 10.0 nmol/L, independent of calcium channel modulation.\textsuperscript{18,32} This antioxidant activity of amlodipine is attributed to both its high lipophilicity and a chemical structure that facilitates proton-donating and resonance-stabilization mechanisms that quench the free-radical reaction.\textsuperscript{18} By inserting to a location in the membrane near conjugated double bonds, highly lipophilic CCBs are capable of donating protons to lipid peroxide molecules, thereby blocking the peroxidation process (Figure 2). The remaining unpaired free electron associated with the drug molecule can be stabilized in resonance structures associated with the dihydropyridine ring, as previously described in detail.\textsuperscript{18} The reaction that describes the antioxidant effects of the dihydropyridine (DHP) CCB is as follows, in which LOO● represents a lipid peroxide molecule: 

\[
\text{LOO}\circlearrowleft + \text{DHP} \rightarrow \text{LOOH} + \text{DHP}\circlearrowright
\]

The antioxidant activity of amlodipine was also observed in vivo in various animal models, including nonhuman primates, thus revealing an important antiatherogenic mechanism of action for this compound.\textsuperscript{33,34} Antioxidant activity has also been reported for other CCBs,\textsuperscript{26,33,34} including lacidipine, a compound with which activity was observed in preclinical models of atherosclerotic disease, as well as in hypertensive patients, in whom a beneficial effect on carotid intima-media thickness and number of plaques was demonstrated.\textsuperscript{12}

![Figure 2. Schematic illustration of the antioxidant activity of a lipophilic CCB. Propagation of free radicals through the membrane lipid bilayer is attenuated in the presence of the CCB because of the proton-donating and resonance-stabilization mechanisms that quench free-radical reaction.](image-url)

**SMC Membrane Remodeling After Cholesterol Enrichment**

Free cholesterol is an abundant lipid component of the cell plasma membrane, where it modulates packing of phospholipid molecules, thus regulating membrane lipid dynamics and structure.\textsuperscript{21,33} The cholesterol molecule is oriented in the membrane such that its long axis lies parallel to the phospholipid acyl chains, increasing order in the upper acyl-chain region of the membrane while decreasing packing constraints at the terminal methyl groups.\textsuperscript{35} During atherogenesis, however, increasing levels of cellular cholesterol lead to its elevation in the plasma membrane, resulting in the formation of distinct cholesterol microdomains that can be characterized by small-angle x-ray diffraction.\textsuperscript{22,23,36}

In VSMCs obtained from atherosclerotic plaques, Ca\textsuperscript{2+} transport mechanisms and basal intracellular Ca\textsuperscript{2+} levels are disrupted as a result of increased membrane cholesterol content.\textsuperscript{37} These changes have important consequences for atherosclerosis, because calcium participates directly in signaling transduction pathways that promote SMC proliferation and migration, among other changes. In endothelial cells, excessive membrane cholesterol incorporation during hyperlipidemia interferes with active-transport mechanisms for amino acids, including L-arginine. As a result, activation of endothelial NO synthase (eNOS) leads to overproduction of superoxide from oxygen, the alternative product of NOS content when quantities of L-arginine are insufficient.\textsuperscript{38}

In models of atherosclerosis, systematic changes in the cholesterol content of vascular cell membranes have been measured and correlated with cholesterol microdomains.\textsuperscript{21} In these studies, the effects of cholesterol enrichment on the molecular dimensions and lipid organization of plasma membranes derived from SMCs grown in vitro and those obtained from an intact animal model were remarkably consistent. Under atherosclerosis-like conditions, prominent cholesterol domains with a unit-cell periodicity of 3.4 nm could be observed in SMC plasma membranes as free cholesterol levels in the membrane increased as a function of elevated...
serum cholesterol levels. In both model and biologic membranes, oxidized cholesterol derivatives also formed domains within the membrane lipid bilayer with distinct structural width characteristics.

After incubation with smooth muscle plasma membranes isolated from atherosclerotic aorta segments, amlodipine reversed the structural changes associated with cholesterol enrichment. Membrane bilayer swelling was attributed to the accumulation of unesterified cholesterol in the diseased artery, an atherogenic stimulus that leads to increased SMC proliferation owing to the production of growth factors. This biophysical effect of amlodipine on membrane structure was independent of calcium channel modulation and can be attributed to its relatively high lipophilicity, mediated by its charged aminoethoxy function.

**Stimulation of NO Synthesis by Amlodipine**

Circumstantial evidence, including slow onset and long duration of action, suggested that amlodipine had pharmacologic properties distinct from other calcium channel antagonists, particularly nifedipine. In addition, a number of studies have suggested that amlodipine inhibits platelet aggregation. In vivo, amlodipine-induced vasodilation was reduced through inhibition of NO synthesis (with use of a substituted arginine molecule) and amlodipine has been shown to increase peripheral and coronary blood flow. Changes in shear stress are thought to be important in the regulation of NO production: whenever blood flow changes, so do shear and NO release. Therefore, all vasoactive drugs have the potential to release NO because of changes in physical forces that distort the endothelial cell. Potential mechanisms through which amlodipine might stimulate the production of NO are represented in Figure 3.

To determine whether amlodipine promotes the release of NO in the absence of changes in shear or blood flow, Zhang and Hintze measured nitrate release from coronary microvessels harvested from dogs. In vitro, amlodipine, unlike nifedipine or diltiazem, caused a dose-dependent release of nitrate, the hydration product of NO. Studies on the ability of NO to regulate tissue oxygen consumption through interactions with cytochrome oxidase in the mitochondrial electron-transport chain indicated that amlodipine released NO in the heart from the mouse, rat, hamster, dog, nonhuman primate, and human. Amlodipine also releases NO in the canine kidney and skeletal muscle in vitro. The effects of amlodipine on both nitrite release and the NO-dependent regulation of cardiac oxygen consumption were reduced by inhibition of NO synthesis. The release of nitrite or inhibition of tissue oxygen consumption was similar in magnitude to those caused by the 3 ACE inhibitors captopril, enalapril, and ramiprilat. The ability of amlodipine to release NO was unexpected, because (1) NOS is a calcium-dependent enzyme and amlodipine should reduce intraendothelial cell Ca2+ and (2) there are no L-type calcium channels on endothelial cells for amlodipine to block.

NO is produced by 3 distinctly different enzymes: (1) eNOS, (2) inducible NOS (iNOS), and (3) neuronal NOS (nNOS). Loke et al sought to determine whether amlodipine-derived NO regulated oxygen consumption in the heart from eNOS−/− mice. Amlodipine, like ramiprilat, had no effect on oxygen consumption in the eNOS−/− mouse heart. In rats chronically treated with an NOS inhibitor to cause hypertension, amlodipine caused an upregulation of eNOS. These data directly linked amlodipine to stimulation of the endothelial isofrom of NOS.

**Role of Kinins in NO Production**

ACE inhibitors release NO, not by modifying the conversion of angiotensin I to angiotensin II, as their name implies, but rather by their ability to inhibit kininase II, the enzyme that breaks down bradykinin. In cardiac tissues from a number of species, N^0-nitro-L-arginine methyl ester (L-NAME) or HOE-140, a bradykinin-2 (B2-kinin) receptor antagonist (icatibant), blocked the ability of amlodipine to reduce cardiac oxygen consumption. Importantly, the effects of amlodipine were also blocked by a number of serine protease inhibitors in the human heart that prevent the local formation of kinins, including dichloroisocoumarin, soybean trypsin inhibitor, and aprotinin. Neutral endopeptidase inhibitors also prevent the breakdown of kinins and enhance NO production.

Amlodipine had no effect on oxygen consumption in the heart from B2-kinin receptor−/− mice, directly linking amlodipine to local kinin production and NO release. This transduction mechanism, from local kinin production to the B2-kinin receptor to NO production, was thought to be novel but has recently been implicated as the mechanism of flow-mediated dilation in the rat carotid artery. In that study, flow-mediated dilation was reduced in the carotid artery from tissue kallikrein−/− and B2-kinin−/− mice, indicating an important role for local kinin formation. Moreover, in the tissue kallikrein+/− carotid artery, flow-mediated dilation was blocked by an NOS inhibitor and HOE-140. Another CCB, benidipine, releases NO, and a portion of its biologic and therapeutic activity is NO dependent. A number of innovative studies hypothesized that benidipine increases the release of kallikrein. Unlike verap-
amil and nifedipine, amlodipine dilated the rabbit femoral artery through an NO-dependent mechanism, and this resulted in NO\textsubscript{(nitrate plus nitrite)} production. The dilation was mediated by local kinin formation and the B\textsubscript{2}-kinin receptor, because it was also blocked by HOE-140.\textsuperscript{52} Clevidipine has been reported to release NO through a kinin-mediated mechanism.\textsuperscript{63} Taken together, these data suggest that (1) there is a local kinin system in blood vessels; (2) activation of the B\textsubscript{2}-kinin receptor stimulates NO production; and (3) this is the mechanism through which amlodipine releases NO.

**Enantiomer-Specific Release of NO by Amlodipine**

Given that there are no L-type calcium channels on endothelial cells and that amlodipine activates eNOS, there must be a mechanism independent of its CCB properties whereby amlodipine releases NO. Like many other CCBs,\textsuperscript{64} amlodipine is a racemic mixture of 2 enantiomers, designated S\textsuperscript{−} and R\textsuperscript{+}. The CCB properties are localized in the S\textsuperscript{−}-enantiomer, whereas the R\textsuperscript{+}-enantiomer has no known biologic effect. The R\textsuperscript{+}-enantiomer of amlodipine caused a dose-dependent increase in nitrite production in canine coronary microvessels and a reduction in cardiac oxygen consumption that was blocked by L-NAME and HOE-140.\textsuperscript{65} The S\textsuperscript{−}-enantiomer had no effect on NO release. In the same study, nitrendipine also released NO; this agent is also an enantiomeric mixture.\textsuperscript{65}

**Role of Angiotensin Receptors in the Release of NO by Amlodipine**

Because the R\textsuperscript{+}-enantiomer of amlodipine does not interact with the L-type calcium channel, what is the receptor for the R\textsuperscript{+}-enantiomer? In 1995, Seyedi et al\textsuperscript{66} showed that activation of the angiotensin II receptor activated local kinin production to stimulate NO formation. The investigators used a specific inhibitor of the angiotensin II type 2 receptor (AT\textsubscript{2}), PD123319, to delineate the transduction mechanism responsible for the release of NO. Recent data suggest that the ability of the R\textsuperscript{+}-enantiomer of amlodipine to release NO was blocked by the AT\textsubscript{2} receptor blocker PD123319 but not by the angiotensin II type 1 (AT\textsubscript{1}) receptor blocker losartan.\textsuperscript{67} However, the ability of the R\textsuperscript{+}-enantiomer of amlodipine to reduce cardiac oxygen consumption still occurred in the AT\textsubscript{2}/−/- mouse heart and was still blocked by the putative specific AT\textsubscript{1} antagonist PD123319. This paradox suggested that PD123319 might not be as specific as earlier believed and led to the conclusion that a receptor closely related to the AT\textsubscript{2} receptor might be important in the control of NO release by amlodipine.\textsuperscript{67}

**Implications of the Release of NO by Amlodipine for the Treatment of CVD**

There are 2 reasons why the ability of amlodipine to release NO is important to the understanding of CVD. The first is that the evolution of diseases characterized by a reduction in NO production might be modified in part by restoring NO production. A reduction in NO production or the biologic activity of NO contributes to the development of heart failure,\textsuperscript{68} diabetes,\textsuperscript{69} atherosclerosis, and hypertension.\textsuperscript{70} In states where eNOS is downregulated, the activity of the enzyme can be enhanced, and drugs such as amlodipine, other CCBs, and ACE inhibitors might confer benefit by restoring NO production. The second reason why understanding the mechanism by which amlodipine releases NO is important is that it provides a rationale for combination therapies. For instance, statins upregulate eNOS.\textsuperscript{71} If statins can restore eNOS protein and other drugs such as amlodipine can stimulate eNOS, then the drugs would have a synergistic effect. In this vein, amlodipine and an ACE inhibitor caused a greater NO-dependent reduction in tissue oxygen consumption in the heart from rats treated with statins than in the heart of untreated rats.\textsuperscript{46} In addition, the production of nitrite in coronary microvessels from canine and human hearts and the NO-dependent regulation of oxygen consumption in explanted human hearts were greater when amlodipine was combined with an ACE inhibitor.\textsuperscript{72,73} The combination was found to be more than additive, indicating a true synergism.\textsuperscript{72} This can be explained through an understanding of the role of kinins in the mechanism of action of both drugs. ACE inhibitors block the breakdown of kinins, and amlodipine stimulates kinin formation by activating or releasing kalikrein. There might even be a synergism between mechanical cardiac support and amlodipine in the regulation of NO production in the human heart.\textsuperscript{74} Finally, there might be synergism between various physiologic states in which eNOS is upregulated, such as exercise\textsuperscript{75} or pregnancy,\textsuperscript{76} and the ability of amlodipine to release NO.

**Involvement of VSMC Growth/Proliferation in Atherosclerosis**

**Effects of CCBs on Signaling Pathways**

Under physiologic conditions, VSMCs are located in the media of the vessel wall, where they exhibit the contractile phenotype; they do not proliferate or perhaps do so at only a very low rate. By contrast, under pathologic conditions such as atherosclerosis, VSMCs display a proliferative/secretory phenotype; ie, the cells proliferate and move into the neointima, where they secrete matrix components. VSMC proliferation can be considered a key event in atherogenesis. Thus, compounds that interfere with the machinery of cell proliferation can be considered potential drugs of interest for the treatment of atherosclerosis.

VSMCs express a large number of voltage-dependent calcium channels that actively participate in the responsiveness to various agonists through the control of intracellular Ca\textsuperscript{2+} homeostasis,\textsuperscript{77,78} and Ca\textsuperscript{2+} movements are undoubtedly involved in cell-cycle initiation/progression.\textsuperscript{79,80} Therefore, it is not unexpected that the different classes of CCBs, including the dihydropyridines, do inhibit VSMC proliferation.\textsuperscript{81} As an example, nifedipine inhibited VSMC proliferation in vitro and suppressed intimal thickening caused by balloon angioplasty–induced injury in rats in vivo.\textsuperscript{82} Amlodipine is a potent inhibitor of VSMC proliferation and migration in vitro.\textsuperscript{83,84} In VSMCs isolated from rat aortas or human internal mammary arteries, amlodipine inhibited DNA synthesis elicited by serum, thrombin, and basic fibroblast growth factor stimulation, and the drug exerted its antiproliferative action early on in the G\textsubscript{1} phase of the cell cycle.\textsuperscript{84} In vivo inhibition by
nifedipine of VSMC proliferation induced by balloon catheter injury also occurs at an early stage of the cell cycle. The mechanism underlying the inhibitory effect of CCBs on VSMC growth is still poorly understood; recent data obtained with amlodipine and several other dihydropyridines have indicated, however, that a more likely mechanism is the drugs’ inhibition of expression of early growth-response genes, including c-myc, c-fos, and c-jun. Considering the high selectivity of amlodipine for the vasculature (versus the heart), one might suppose that its antiproliferative action on VSMCs might contribute to its antiatherogenic effect. Thus, it is important to unravel the mechanism whereby amlodipine attenuates VSMC growth/proliferation. Some key mechanisms that are likely involved in the overall effect of amlodipine are considered next.

Effect on Calcium Signaling
With use of the enantiomers of lercanidipine and verapamil, it has been shown that their inhibition of VSMC proliferation is independent of their CCB activity. Similarly, experiments with rat aortic VSMCs suggested that in contrast to nifedipine, amlodipine-elicited inhibition of serum-, thrombin-, and basic fibroblast growth factor–triggered VSMC proliferation involved mechanisms independent of CCB properties. In support of this hypothesis, amlodipine has been reported to inhibit thrombin-induced Ca^2+ mobilization from thapsigargin-sensitive, internal Ca^2+ stores; this effect could not be obtained with isradipine, diltiazem, and verapamil. An interaction between amlodipine and/or its receptors and the Ca^2+ pump of the sarcoplasmic reticulum (SERCA) could account for these observations and would be of physiopathologic relevance, because a causal relation has been established between Ca^2+ influx, SERCA Ca^2+ ATPase activity/expression, and the control of cell-cycle progression from G1 to S. On the other hand, amlodipine also exerts its CCB effects on VSMCs. Thus, in cultured human VSMCs, the inhibition of L-type calcium channels by amlodipine (or isradipine) decreased basic fibroblast growth factor–induced DNA synthesis, and this was associated with inhibition of expression of early growth-response genes. 

Effect on NO Production/Release and Antioxidants
Pharmacologic and gene-transfer studies or experiments with transgenic models have shown that NO inhibits VSMC proliferation in vitro and in vivo (reviewed in Jeremy et al). The mechanism of action involves both cGMP-dependent and -independent pathways and results in cell-cycle arrest. As thoroughly documented in the present review, amlodipine markedly increased the release of NO in the coronary microvessels of dog hearts, whereas nifedipine and diltiazem were ineffective. The effect of amlodipine seems to be dependent on a kinin-mediated mechanism and independent of the drug’s L-type calcium channel activity. In another animal model, benidipine has been shown to inhibit intimal thickening by increasing vessel eNOS expression and NO production.

Oxidation of LDL is a key process in atherogenesis. The regulation of VSMC growth by oxidized LDLs operates through the activation of the Ras/Raf/MEK/MAPK signaling pathway by a Pertussis toxin–sensitive, G protein–coupled receptor. Moreover, most of the CCBs, perhaps with the exception of diltiazem, exert antioxidant properties in vitro and reduce the LDL oxidation process. Antioxidants potently reduce VSMC proliferation and, as reported in detail earlier, amlodipine exhibits particular antioxidant properties.

Other Effects
Matrix metalloproteinases (MMPs) actively participate in VSMC proliferation and vessel-wall remodeling. Recently, various CCBs, including amlodipine, have been reported to modulate MMP activity. Although the mechanisms of action involved are still unclear, CCBs might act not only on MMP activity/expression but also on transcription of the tissue inhibitor of metalloproteinases (TIMPs). It has also been reported that several CCBs, including amlodipine, prevented induction of the hydroxymethyl glutaryl coenzyme A reductase gene and enhanced platelet-derived growth factor–BB induced LDL receptor gene and expression. As for statins, this could affect VSMC responsiveness through interaction with the Rho signaling pathway; such a hypothesis remains to be investigated.

Therefore, in addition to inhibition of voltage-gated L-type calcium channels, all of the mechanisms mentioned earlier (and summarized in Figure 4) contribute to amlodipine’s inhibition of agonist-induced VSMC reactivity and proliferation, likely through inhibition of growth/proliferation gene expression. The particular characteristics displayed in text.
by amlodipine likely contribute to its potential antiatherogenic properties.

**Conclusions**

Initial studies of the mechanisms of action of L-type CCBs indicated that these drugs were potent and selective vasodilators because of their ability to reduce intracellular SMC Ca\(^{2+}\) concentration. More recent studies suggest that alterations in gene expression and calcium-regulated calcium release also inhibit VSMC proliferation. In addition to these actions, increasing basic and clinical data suggest that there are non–calcium-related pleiotropic actions of CCBs. Amlodipine can regulate membrane fluidity and cholesterol deposition, stimulate NO production to recruit its biologic actions, act as an antioxidant, and regulate matrix deposition. Recognition of the ancillary actions of amlodipine is important for understanding the agent’s mechanisms of action and the pathologic mechanisms underlying disease; it is also vital for determination of the rational use of this and similar agents in the treatment of CVD. In some experimental studies, the concentration of amlodipine might be higher than are plasma levels in patients. The exact clinical significance of these actions remains to be determined. However, a fuller understanding of these pleiotropic actions might offer insight into why some drugs confer benefit in certain diseases and not in others. Finally, studies of the pleiotropic actions of amlodipine might lead to a better understanding of the mechanism of action of all CCBs.

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**References**


20. Mason RP, Herberge LG, Tulenko TN. Cholesterol enrichment during dietary atherosclerosis alters smooth muscle plasma membrane width and structure: evidence for reversal by the 1,4-di-hydropyridine amlo-


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