Editorial

Translocation of endothelial nitric oxide synthase: Another feat of amlodipine, a cardiovascular jack-of-all-trades

Bernd Mayer

Department of Pharmacology and Toxicology, Karl-Franzens University Graz, Univ.-Platz 2, A-8010 Graz, Austria

Received 29 May 2006; accepted 2 June 2006

Available online 9 June 2006

See article by Batova et al. [12] (pages 478–485) in this issue.

The antihypertensive drug amlodipine (Norvasc®) is a long-acting, third-generation Ca\(^{2+}\) channel blocker that has been shown to limit the progression of atherosclerosis and to decrease the incidence of cardiovascular events in humans [1]. It is generally well tolerated and often used in combination with diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists and statins [2–4]. Amlodipine exhibits nanomolar affinity to dihydropyridine as well as benzothiazepine and phenylalkylamine binding sites of voltage-dependent L-type Ca\(^{2+}\) channels [5]. It is several-fold more lipophilic than the first- and second-generation Ca\(^{2+}\) channel blockers and adopts a well-defined position in the bilayer membrane [6]. It has been suggested that partitioning of amlodipine to membranes results in beneficial modification of the structure of cholesterol-rich membranes of vascular smooth muscle cells, an effect that might contribute to atheroprotection [7].

Besides the well-documented inhibition of L-type Ca\(^{2+}\) channels, amlodipine exerts additional effects that contribute to the improvement of vascular function in hypertension and ischemic heart disease [8]. There is both in vitro and in vivo evidence indicating that dihydropyridines including amlodipine act as antioxidants preventing the progression of atherosclerosis by interfering with lipid peroxidation. However, amlodipine has pharmacological properties distinct from other Ca\(^{2+}\) channel blockers. Probably most relevant to the antihypertensive and antiplatelet effects of the drug is the release of endothelium-derived nitric oxide (NO). This could be a consequence of an antioxidant, superoxide dismutase-like activity of amlodipine, but a recent study suggests the opposite, i.e. that its apparent superoxide scavenging activity may be due to NO release [9]. Alternative explanations for the NO-dependent action of amlodipine include inhibition of ACE-catalyzed breakdown of bradykinin, resulting in enhanced kinin-mediated endothelium-dependent vasodilatation [10], and changes in the phosphorylation status of endothelial nitric oxide synthase (eNOS) at Ser1177 and Thr495 [11]. The latter effect would mimic agonist-triggered eNOS activation by bradykinin, VEGF, or shear stress.

The study by Batova et al. in this issue [12] reveals yet another surprising action of amlodipine that may explain how the drug stimulates release of endothelium-derived NO. It is well documented that eNOS targets to membrane fractions via N-terminal myristoylation and palmitoylation and, within the plasma membrane, to a subclass of lipid rafts, the caveolae, which form a cholesterol-rich microdomain for signaling complexes at the cell surface [13]. In resting endothelial cells, eNOS forms an inhibitory complex with the main structural protein of endothelial caveolae, caveolin-1. The eNOS–caveolin protein complex dissociates in response to Ca\(^{2+}\) mobilizing agonists, which promote calmodulin binding to eNOS, resulting in enzyme activation and increased susceptibility of the protein to phosphorylation [13]. As predicted by this concept, in vivo delivery of the caveolin-1 scaffolding domain to mice results in significant inhibition of endothelial NO synthesis and endothelium-dependent relaxation [14], whereas blood vessels of caveolin knockout mice are hypersensitive to acetylcholine [15]. In the current study, Batova et al. [12] report that treatment of cultured bovine aortic endothelial cells with increasing concentrations of amlodipine (0.1–10 nM) resulted in dissociation of eNOS from caveolin, whereas verapamil (10 μM) and nifedipine (1 μM) had no effect. Since the Ca\(^{2+}\)...
chelator BAPTA did not prevent eNOS translocation, the action of amlodipine appears to be distinct from known triggers of eNOS–caveolin dissociation, which are all Ca$^{2+}$-dependent. The authors speculate that amlodipine may act through a steric membrane effect rather than through initiation of specific signaling cascades. This would imply that the drug promotes dissociation of caveolin binding proteins other than eNOS as well. Future studies are warranted to clarify this issue.

The amlodipine-triggered dissociation of eNOS from caveolin was accompanied by approximately 1.5- and 2-fold increases in NO release from cells stimulated with bradykinin and VEGF, respectively. These results confirm that eNOS activation in response to both Ca$^{2+}$-dependent (bradykinin) and so-called Ca$^{2+}$-independent agonists (VEGF) is significantly diminished by caveolin binding. One might expect that basal eNOS activity becomes also increased upon disruption of the inhibitory eNOS–caveolin complex, but the authors observed no effect of amlodipine in the absence of agonists. Thus, amlodipine-induced translocation appears to predispose eNOS for efficient stimulation by agonists without affecting enzyme activity per se. In striking disagreement to this finding, Fleming and coworkers reported on a rapid, approximately 3-fold increase in basal NO release from resting endothelial cells in response to amlodipine. This discrepancy might be related to the 100-fold higher concentration of amlodipine (1 μM) used by the Fleming group [11].

The authors took great efforts to make sure that the effect of amlodipine on NO release was indeed a consequence of caveolin dissociation from eNOS. Scavenging of superoxide and inhibition of ACE-catalyzed kinin degradation were considered as potential alternatives but excluded in carefully performed control experiments using bovine serum as source of ACE, lisinopril as established ACE inhibitor, and PEG-SOD as superoxide scavenger. Arguably the most informative results were obtained with endothelial cells transfected with a caveolin-1 plasmid in order to artificially increase caveolin levels. The authors show that excess caveolin largely prevents the effect of amlodipine on NO release stimulated with bradykinin or VEGF. These results reinforce the concept that the release of NO from endothelial cells in response to both Ca$^{2+}$-dependent and Ca$^{2+}$-independent agonists is determined by the equilibrium between caveolin-bound and caveolin-free eNOS, and demonstrate that amlodipine facilitates eNOS activation by shifting this equilibrium towards the caveolin-free state.

The current study adds-up to three the potential mechanisms by which amlodipine could induce endothelial NO synthesis: (i) inhibition of ACE-catalyzed bradykinin breakdown [10], (ii) altered phosphorylation status of eNOS via inhibition of protein kinase C [11], and (iii) unclamping of eNOS from the inhibitory caveolin complex [12]. The relative contribution of the different mechanisms to the overall effect of amlodipine is hard to judge. Based on the results obtained by Batova et al., the contribution of ACE inhibition appears to be minimal, but dissociation from caveolin combined with altered phosphorylation at Ser1177 and Thr495, at least at higher concentrations of amlodipine, may synergistically facilitate agonist-induced eNOS activation. Transfection of cells with appropriate eNOS mutants combined with caveolin knock-down and overexpression might be considered as a promising approach to pin down the molecular mechanisms by which amlodipine exerts its stunning effects on NO release from vascular endothelial cells.

Batova et al. have not addressed the potential in vivo relevance of their data. Amlodipine binds with an affinity constant of 1.7 nM to rat cardiac membranes [5] and inhibits Ca$^{2+}$-induced contraction of rat aorta with an IC$_{50}$ of 1.9 nM [16]. This is well within the range of concentrations reported here to trigger eNOS translocation, suggesting that this process may occur upon in vivo application of the drug. Thus, the authors may have discovered the first small molecular weight molecule that is able to promote dissociation of the inhibitory eNOS–caveolin complex in vivo. Their results not only provide an elegant confirmation of the physiological importance of the eNOS–caveolin interaction, but also pave the way for the development of a new class of drugs that improves vascular function by unclamping eNOS from the inhibitory caveolin complex.

References


