Dihydropyridine Calcium Antagonist-Induced Modulation of Endothelial Function: A Review

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INTRODUCTION

The vasorelaxing properties of calcium antagonists have been well established in both experimental and clinical settings during the past three decades. Fleckenstein et al. (13) first postulated a blockade of calcium channels by verapamil, a calcium antagonist. The first therapeutically useful dihydropyridine (DHP), nifedipine, was synthesized by Bossert and Vater (9), and formed the basis for a novel class of substances with either calcium antagonistic or agonistic properties. Since then, DHPs have been well characterized (16) and so-called second or third generation dihydropyridines with improved properties were developed. These compounds were either less light sensitive, less cardiodepressant, caused less reflex tachycardia, or had a longer duration of action (34). They all bind (10) to the $\alpha_1$ subunit (21) of voltage-operated L-type calcium channels in vascular smooth muscle cells, thereby inhibiting calcium influx and causing vasorelaxation (the major mechanism of their therapeutic action in the treatment of coronary heart disease and hypertension).

In 1990 Kojda et al. (27) studied the vascular selectivity of DHPs and found that DHP-induced vasorelaxation can be modulated by vascular endothelium (27). This was surprising since macrovascular endothelial cells do not express L-type calcium channels (1,11), the specific target of DHPs. It is known that endothelium release factors that control vascular relaxation and contraction, thrombogenesis, fibrinolysis, and platelet activation (30). The major endothelial factor is nitric oxide (NO). It was suspected that interference with endothelial function could be at least partly responsible for some of the vascular effects of DHPs. This review focuses on the mechanism of DHP-induced modulation of endothelial NO release.

FUNCTIONAL STUDIES

NO is a small diffusible molecule with a variety of biological functions (15,32). It was first discovered by Furchgott et al. (17) as an “endothelium-derived relaxing factor” and
later identified as NO by Palmer et al. (35) and Ignarro et al. (22) in 1987. Endothelium-derived NO causes vascular relaxation via cGMP-mediated mechanisms (30) and, moreover, inhibits platelet aggregation and leukocyte adhesion (2).

The first indication that DHPs may alter endothelial NO formation was obtained in studies in which methylene blue was used to block guanylate cyclase (GC) (27), the specific target of NO (15,32). In PGF$_{2\alpha}$-precontracted porcine coronary and basilar vessels methylene blue was observed to shift the nitrendipine concentration-response curve to the right (toward higher concentrations of nitrendipine) (27), an effect which could have been achieved also by denuding vessels of endothelium (28). The results of both studies suggested that a component of nitrendipine-induced vasorelaxation was dependent on the presence of intact endothelium and, therefore, probably on the formation of NO. When a more specific tool to block NO synthesis, N-nitro-L-arginine (NNA), a competitive inhibitor of the endothelial nitric oxide synthetase (NOS) (15,32), became available, the same experiments were repeated by Guenther et al. (19,20). Again, the concentration-response curve of nitrendipine was shifted to the right. It appeared that up to 30% of the vasorelaxation caused by nitrendipine could be attributed to NO release (19) (Fig. 1).

These results were duplicated using another DHP calcium antagonist, nifedipine (25), at even lower concentrations than those of nitrendipine. The comparison of the concentration-response curves for the two DHPs in these experiments revealed that the NO-dependent vasorelaxation occurs at lower concentrations than the direct effect of the DHP calcium antagonists on vascular smooth muscle (25). Other investigators (39), using

FIG. 1. Nitrendipine (NTD)-induced relaxation of precontracted (by PGF$_{2\alpha}$) porcine coronary arteries (left panel). The concentration-response curve is significantly shifted to higher nitrendipine concentrations by L-N-nitro-arginine (NNA), an inhibitor of the endothelial NO synthase (n = 6). The right panel shows the difference between these curves indicates that up to 30% of the relaxation is dependent on the endothelial NO formation (25).
precontracted rat aorta, confirmed the existence of a NNA-sensitive component in the vasorelaxant effect of different DHPs (lacidipine, isradipine, nisoldipine). Such a component has not been found, however, in canine femoral arteries using nifedipine, whereas a novel DHP antagonist, S 11568 or \([(+/−)-2(2-[2-(aminoethoxy)ethoxyl]methyl)4-(2',3'-dichlorophenyl)3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine], produced an endothelium-dependent relaxation in this model (40).

In a more complex model, an isolated perfused guinea pig heart, nifedipine and nitrendipine increased flow of perfusate, an effect that was blocked by NNA (25). In this model nifedipine was more potent than nitrendipine. This difference in potency appeared to be due to a more pronounced NO-releasing component of nifedipine, since after blockade with NNA both substances were equipotent. Such a difference in potency was also observed in experiments with the mesenteric microvessels of guinea pigs in which diameter changes were videometrically measured (12). Also in these experiments nifedipine, nitrendipine, and nisoldipine—in decreasing order of potency—exerted endothelium-dependent vasorelaxation. More recently, the DHP calcium antagonist benidipine was found to increase coronary blood flow in open-chest dogs. This coronary vasodilator effect was partially blocked by the NOS inhibitor N-monomethyl-L-arginine (L-NAME) (24). The increase in coronary blood flow was accompanied by an increase in the formation of nitrate and nitrite, the metabolic products of NO. cGMP levels in coronary arteries of the ischemic myocardium were also increased, indicating that NO formation was enhanced.

**NO RELEASE**

Since at the time of the early functional studies no techniques had been established to measure NO directly, the indirect hemoglobin assay (23) was used to study the release of NO from various porcine vessels (18,19). Nitrendipine induced a concentration-dependent NO release from porcine coronary, basilary, and tail arteries, and this effect was blocked by NNA. Furthermore, in bovine aortic endothelial cell cultures stimulated with nitrendipine, the enhanced release of NO was abolished by NNA (38). More recently, using a specific electrochemical method to measure NO directly, Berkels et al. (3,7) found that nifedipine induced a concentration-dependent NO release from native cells of porcine coronary arteries and that NO release was blocked by NNA (Fig. 2). This effect had been observed already at nanomolar concentrations of nifedipine (similar to those required for the above mentioned functional effects). The NNA-induced inhibition of relaxation could be partly restored by the NO precursor L-arginine (3,7). A comparison between the NO-releasing effects of nifedipine and nitrendipine revealed that, at least at therapeutic concentrations, nifedipine was more potent in releasing NO than nitrendipine (4). The nifedipine-induced NO release was also seen in porcine endothelial cell cultures. In this preparation, Bay K 8644, a calcium agonist, showed a similar, although less pronounced, effect (4). These findings were in agreement with earlier results obtained using the hemoglobin assay and native endothelial cells (19). In contrast to these results, Mühge et al. (33) were unable to detect an effect of nifedipine on NO release in endothelial cell cultures. Since these authors used a chemiluminescence assay, it is possible that NO reacted with radicals and was no longer detectable. It was reported more recently that amlodipine, but not nifedipine, increases release of NO from canine coronary microvessels (42). These investigators measured nitrate formation rather than using direct electro-
chemical measurements so the discrepancy may have been due to a lesser sensitivity of this assay. Another puzzling finding in this study was the identical rate of NO formation (per mg protein) in micro- and macrovascular samples, although the number of endothelial cells in these samples should have been appreciably different.

**SIGNAL TRANSDUCTION**

The increased release of NO might be due either to an enhanced formation of NO via the endothelial NOS or to a diminished degradation of NO. Preliminary observations indicate the participation of both mechanisms.

The macrovascular endothelial cells are lacking L-type calcium channels (1,11). The signal transduction of the DHP-induced NO release must, therefore, be different in these cells from the conventional mechanism of action of these compounds in cells which express L-type calcium channels (e.g., smooth muscle cells). Since the constitutive endothelial NOS is calcium/calmodulin-dependent (15,32), we first had to determine whether DHPs alter the intracellular calcium level in endothelial cells. In suspended bovine aortic endothelial cells nitrendipine produced a concentration-dependent increase in intracellular calcium (38). Contrary to the receptor-mediated rapid effect of bradykinin, the

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**FIG. 2.** This figure shows the concentration-dependent nifedipine (NIF)-induced NO release in porcine coronary arteries (n = 6) which is completely blocked by preincubation with L-N-nitro-arginine (NNA) and partly restored by the NOS substrate L-arginine (ARG) (3). The shaded bars show the concentration-dependent increase in the intracellular calcium induced by nifedipine (7).
shape of the DHP-induced calcium signal was characterized by a slow onset with a sustained plateau phase that may indicate an increased influx of calcium rather than its release from the intracellular stores. This conclusion was supported by the observation that the effect of nitrendipine was completely prevented by removal of the extracellular calcium. This observation was confirmed in porcine endothelial monolayers (4,5,7) (Fig. 2) in which nifedipine induced the same, although more potent, effect than nitrendipine. This finding was congruent with the above mentioned functional effects and the effects of these drugs on NO release. In this preparation Bay K 8644, a calcium agonist, induced calcium influx, similar to that produced by calcium antagonists (7). It is possible that stress operated calcium channels might have mediated the increased calcium influx. The blockade of these channels with gadolinium inhibited DHP-induced calcium influx in suspended bovine endothelial cells (38), although the channel selectivity of gadolinium in this model still remains to be evaluated.

It is also conceivable that kinases might play a role in the DHP-induced transduction of calcium signals in endothelial cells. The pretreatment of endothelial cells with inhibitors of tyrosine kinases (genistein) or protein kinase C (H7, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine) diminished $[Ca^{2+}]_i$ and the NO signal elicited by nifedipine (5,8). It has been speculated also that kinin receptor-dependent processes might be involved. Zhang et al. (42) showed that amlodipine-induced NO formation in endothelium is blocked by a synthetic peptide with bradikinin$_2$-receptor–antagonist activity (HOE 140) and partly abolished by dichloroisocoumarin (DCIC), a serine protease inhibitor that blocks kinin-forming enzymes. Additionally, it has been found that DHPs possess antioxidant properties (31). DHPs may prolong the short half-life of NO by scavenging reactive oxygen molecules, leading to a longer-lasting and enhanced release of NO.

CONCLUSIONS

The observations reviewed here lead to the conclusion that DHP antagonists cause vasorelaxation by two mechanisms (Fig. 3): directly, by blocking voltage-operated L-type calcium channels in smooth muscle cells; and indirectly, by increasing NO release from the intact endothelium. The latter mode of action is shared with the DHP calcium agonist Bay K 8644 (18). It seems that these effects are linked to increased endothelial calcium levels. The kinases or kinin receptors might also be involved in the signal transduction, although a protective antioxidant action of the DHPs might also play a role.

These findings support the existence of an endothelium-dependent component of DHP-induced vasorelaxation. Since endothelium respond differently to various DHPs, it is conceivable that differences in the “vascular selectivity” of these drugs observed in some experimental and clinical settings (34) are due to the differences in their endothelium-dependent component of vasodilator action. Some of the results reviewed here are still controversial, and “black boxes” still exist in the signal transduction pathway. Some of the results that support an endothelium-dependent component of DHP-induced vascular relaxation were obtained in vitro with rather high concentrations of DHPs. It is possible, however, that the lipophilic DHPs accumulate in vivo in cell membranes in substantially higher local concentrations. Nevertheless, the predominant evidence points to a novel mode of action of DHP calcium antagonists that might represent the missing link in our understanding of the action of these drugs on nonexcitable cells that are lacking L-type
calcium channels. Some secondary effects of DHPs, such as their antithrombotic (6,37), antiproliferative (14,26), and even antiatherosclerotic (14,29,41) properties might be mediated at least in part by their NO-releasing effects (Fig. 3).

SUMMARY

This paper reports on the interactions of various dihydropyridine (DHP) derivatives with endothelial functions in isolated vessels and endothelial cell cultures. Since part of...
the vasorelaxing effect of DHP calcium antagonists is dependent on intact endothelium, which does not possess L-type calcium channels (the specific target of these drugs), an additional mechanism of action may be assumed. This review focuses on the effects of these compounds on endothelial nitric oxide (NO) release. It has been shown that DHP calcium antagonists and agonists increase endothelial NO release. This effect is linked to the elevation of calcium levels in endothelial cells due to increased influx. The signal transduction of this receptor-independent effect seems to involve kinases since inhibition of tyrosine kinases or protein kinase C partly abolished these effects. Modulation of endothelial NO may explain the action of DHPs on nonexcitable cells and appears to be responsible for their antithrombotic and antiatherosclerotic properties.

REFERENCES


