A Number of Marketed Dihydropyridine Calcium Channel Blockers Have Mineralocorticoid Receptor Antagonist Activity

Jessica D. Dietz, Sarah Du, Charles W. Bolten, Maria A. Payne, Chunsheng Xia, James R. Blinn, John W. Funder, Xiao Hu

Abstract—Calcium channel blockers are widely used antihypertensives. Mineralocorticoid receptor antagonists are also used to treat hypertension and heart failure. We report here that a number of widely used dihydropyridine class calcium channel blockers are able to inhibit aldosterone-induced activation of mineralocorticoid receptor. These dihydropyridines varied in the extent of their effect on mineralocorticoid receptor, with nimodipine and felodipine the most potent and amlodipine the least. In contrast, both diltiazem and verapamil, nondihydropyridine calcium channel blockers, had no effect on mineralocorticoid receptor. These dihydropyridines compete with aldosterone for binding and block aldosterone-induced coactivator recruitment to mineralocorticoid receptor. The mineralocorticoid receptor S810L mutant, which is activated by steroidal mineralocorticoid receptor antagonist such as eplerenone, is inhibited by these drugs. Furthermore, nimodipine decreased aldosterone-induced expression of the mineralocorticoid receptor target gene epithelial sodium channel gamma subunit in adrenalectomized rats, demonstrating that dihydropyridine calcium channel blockers can function as mineralocorticoid receptor antagonists in vivo. Molecular modeling indicates that dihydropyridines dock into the ligand binding domain of mineralocorticoid receptor in a consensus pose that partially overlaps with steroidal mineralocorticoid receptor antagonists. Together, our data suggest that, in addition to their calcium channel blocking activity, a number of dihydropyridine calcium channel blockers also have mineralocorticoid receptor antagonist activity at high doses, a finding which may thus prove useful for the design of novel antihypertensive drugs in the future. (Hypertension. 2008;51:1-7.)

Key Words: calcium channel blockers ■ aldosterone ■ mineralocorticoid receptor antagonist ■ dihydropyridine

Hypertension is a widespread public health problem and a major risk factor for cardiovascular and renal disease. Numerous antihypertensive drugs have been developed to lower blood pressure (BP); these drugs target a number of mechanisms and are often used in combination.1,2 Calcium channel blockers (CCBs) are among the most frequently used antihypertensives and are grouped into 2 classes based on the chemical structures: the dihydropyridines such as amlodipine and nifedipine, and nondihydropyridines (diltiazem and verapamil).3 Another heavily targeted mechanism for treatment of hypertension is the renin-angiotensin-aldosterone system. Angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) are among the standard therapies for BP control.4 Mineralocorticoid receptor (MR) antagonists, such as eplerenone, block MR activation and show similar BP lowering efficacy as ACEi or ARB.5,6 Targeting multiple mechanisms provides an advantage in control of hypertension, as most hypertensive patients require 2 or more medications to achieve their BP goal.7 Here we report a surprising finding, that the dihydropyridine CCBs have MR antagonist activity. A number of dihydropyridine CCBs compete for aldosterone binding to the MR ligand binding domain (LBD), block aldosterone-induced recruitment of coactivators, and inhibit aldosterone-induced gene expression. MR antagonist activity of CCBs is restricted to the dihydropyridine class, and the nondihydropyridine CCBs have no effect on MR.

Materials and Methods

Reagents and Cell Culture
CCBs were purchased from Sigma (St. Louis, Mo). Cell culture, transfection, and luciferase assay were performed as described previously.5

MR Scintillation Proximity Binding Assay
Tritium labeled aldosterone (PerkinElmer) was added to insect-cell expressed glutathione-S-transferase (GST)-MR-LBD in the presence of 0.5 mg/well glutathione-coated yttrium silicate beads (GE Health-
and various concentrations of cold competitor compounds. The mixture was incubated on a shaker for 1 hour at room temperature. The bound $^3$H-aldosterone was captured by glutathione coated scintillation beads and detected using Microplate Scintillation Top-Count NXT (PerkinElmer).

**ENaCγ Assay**

Male Sprague-Dawley rats (Harlan Sprague-Dawley Industries, Indianapolis, Ind) were anesthetized with 5% Isoflurane (Baxter Inc) and a bilateral adrenalectomy performed. Animals were maintained on 0.9% NaCl for 3 days to allow recovery for surgery. After an overnight fast, rats were randomly assigned into treatment groups. MR antagonists were dosed either orally or via intraperitoneal injection in suspension. Aldosterone (5 µ/kg, Sigma) was given by subcutaneous injection 30 min postantagonist treatment. Distal colon was collected at 2 h postantagonist dose. RNA was isolated and purified from the frozen colon using the RNAeasy 96 system (Qiagen). ENaCγ expression was detected using Taqman. All procedures in the animal studies have been approved and were conducted in compliance with the Animal Welfare Act Regulations (9 CFR Parts 1, 2, and 3) and the *Guide for the Care and Use of Laboratory Animals* (ILAR, 1996), as well as with all internal company policies and guidelines.

**Docking Studies**

Molecular modeling was conducted using the Schrodinger Suite 2006 (Schrodinger Suite 2006 Induced Fit Docking protocol; Glide version 4.0, Prime version 1.5, Schrodinger LLC). The wild-type MR and S810L docking structures were prepared from the 1.95 Å MR structure with bound corticosterone (PDB: 2A3I)9 and the 1.95 Å S810L crystal structure with bound progesterone (PDB: 2AA6),10 respectively, by removal of waters, truncation of Helix 12 to the C terminus (residues 958 to 984), and capping of Pro957 as the N-methylacetamide. Dihydropyridine ligands were geometry optimized using Jaguar version 6.5 (B3LYP/6p to 31G***/B3LYP/6p to 31G**) to ensure appropriate ring geometries. The induced fit docking protocol11 was used to dock the dihydropyridines and eplerenone into the truncated receptor models, using the default induced fit docking protocol parameters with the exception of using XP scoring for the redock step. No hydrogen bonding or other constraints were used. Pictures were generated using Maestro 8.0.308 (Schrodinger LLC).

**Results**

**Dihydropyridine but Not Other CCBs Inhibit MR Activation by Aldosterone**

In screening for MR ligands, we came across a number of compounds belonging to the dihydropyridine CCBs. As shown in Figure 1A, all the frequently used dihydropyridine CCBs inhibit aldosterone-induced activation of a luciferase reporter driven by MR LBD (fused to a heterologous Gal4 DNA binding domain). The dihydropyridines tested show various inhibitory effects at 10 µmol/L with complete inhibition elicited by nimodipine, felodipine, and nitrendipine, but only about 50% by amlodipine.

A dose response study was followed to obtain the potency of these compounds. The representative curves are shown in Figure 1B and the IC50 values are summarized in Table 1. Consistent with the data shown in Figure 1A, nimodipine, felodipine, and nitrendipine are the most potent MR antagonists with IC50 values ranging from 160 to 450 nmol/L, similar to that of eplerenone in this assay system.12 Amlodipine, the least potent of these compounds, has an IC50 value of 7.4 µmol/L.

The above inhibitory effect was observed at concentrations substantially higher than the affinity for calcium channels,13,14 suggesting a calcium channel independent effect. To completely rule out the possibility that blocking calcium channels contributes to MR inhibition, we tested the 2 nondihydropyridine CCBs, diltiazem and verapamil. As shown in Figure 1C, neither drug has an inhibitory effect on MR at concentrations as high as 20 µmol/L, demonstrating that the MR-inhibitory effect is specific to the dihydropyridine chemical class and not due to an indirect effect via blocking calcium channels.
Dihydropyridine CCBs Compete for Aldosterone Binding to MR LBD

To demonstrate a direct binding of dihydropyridine CCBs to MR, we used a scintillation proximity assay to examine the ability of selected dihydropyridine CCBs to compete for 3H-aldosterone binding to MR LBD. As shown in Figure 2, binding of 3H-aldosterone to MR LBD was inhibited by all 3 compounds in a dose-dependent fashion; again, nimodipine and felodipine appear much more potent than amlodipine, and only slightly less active than eplerenone in competing for binding to MR LBD. The relative affinities of these compounds for MR LBD are consistent with the relative potencies observed in the reporter assay system (Figure 2 and Table 1).

Dihydropyridine CCBs Block the Aldosterone-Induced Interaction With Coactivators

MR, like other nuclear receptors, regulates transcription of target genes via ligand-dependent recruitment of coactivators. Having established that dihydropyridine CCBs bind MR and inhibit aldosterone-induced transcription activation, we further examined whether these compounds modulate the recruitment of coactivators. We have previously shown that the peptide SRC1–4a derived from coactivator SRC1 interacted strongly with MR LBD in the presence of agonist aldosterone, and that the steroidal MR antagonist eplerenone completely blocked this interaction. As shown in Figure 3A, none of the dihydropyridine CCBs induced a significant interaction with SRC1–4a in the absence of aldosterone. When aldosterone is present, these compounds blocked SRC1–4a recruitment (Figure 3B). The degree of inhibition correlates well with the relative potency of these compounds, again with nimodipine, felodipine, and nitrendipine the most active and amlodipine the least. Moreover, when we tested nimodipine at a series of concentrations, the dose response curve is essentially superimposable with that obtained in the

Table 1. Potencies of Dihydropyridine CCBs on MR

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μmol/L)±SE</th>
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<tbody>
<tr>
<td>Nifedipine</td>
<td>0.71±0.08</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>7.40±1.73</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>3.30±0.96</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>Isradipine</td>
<td>1.61±0.39</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>0.45±0.08</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>3.00±0.65</td>
</tr>
</tbody>
</table>

IC50 indicates half effective concentration of inhibition; SE, standard error.
reporter assay (Figure 1B and 3C). Together, these results indicate that these dihydropyridine CCBs are bona fide MR antagonists.

Dihydropyridine CCBs Can Also Antagonize MR Mutant S810L

The MR S810L mutation was identified in patients with early-onset hypertension exacerbated by pregnancy. Progesterone, spironolactone, and eplerenone, which antagonize wild-type MR, partially activate the S810L mutant.\(^8\) We next examined the effect of dihydropyridine CCBs on this mutant receptor. As shown in Figure 4A, none of these CCBs activated the S810L when tested at 10 μmol/L concentration; as expected, eplerenone partially activated the mutant. When they were tested in the presence of aldosterone, all compounds decreased aldosterone-induced activation of the S810L. Nifedipine, isradipine, and nisoldipine showed almost complete inhibition of the activated mutant but not wild-type receptors, whereas nimodipine, which completely inhibited the wild-type receptor, showed only about 90% inhibition of the mutant receptor at 10 μmol/L. These discrepancies reflect the different potencies of these compounds on the mutant receptor (Table 2). The demonstration that these dihydropyridine CCBs inhibit aldosterone activation but had no effect on the basal activity of the S810L mutant indicated that, unlike the steroidal MR antagonists, these dihydropyridine CCBs function as full antagonists on this mutant receptor.

Nimodipine Inhibits the Aldosterone-Induced Expression of the Gamma Subunit of Epithelial Sodium Channel (ENaC\(\gamma\))

All 3 subunits of the epithelial sodium channel are regulated by MR. Both MR and the \(\gamma\) subunit of epithelial sodium channel (ENaC\(\gamma\)) are expressed in the epithelial cells of the distal colon,\(^16\,17\) a classical site where MR regulates electrolyte balance. ENaC\(\gamma\) is induced on injection of aldosterone into the adrenalectomized rats (unpublished data, 2007). We examined the effect of nimodipine on the expression of the ENaC\(\gamma\) gene in the distal colon of adrenalectomized rats. Nimodipine was chosen because it is the most potent MR antagonist among the dihydropyridine CCBs tested, and a literature survey indicated that its plasma concentrations in rat can reach a level considerably above its IC50 value for MR.\(^18\) As shown in Figure 5, aldosterone significantly increased the expression of ENaC\(\gamma\), and this induction can be largely abolished by preadministration of eplerenone. Nimodipine when administered either orally or via intraperitoneal

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**Figure 4.** Effects of dihydropyridine CCBs on MR S810L mutant. The experiments were conducted as in Figure 1 except that the S810L mutant was used, in the absence (A) or presence (B) of 1 nmol/L aldosterone. Dihydropyridines and eplerenone were at 10 μmol/L. No significant activation was observed in the absence of any compound or presence of CCBs. The activity in the absence of any compound was set as 0%. Statistic significance (unpaired \(t\) test) vs aldosterone group in B: amlodipine, \(P<0.01\); nicardipine, \(P<0.003\); all other groups, \(P<0.001\).

**Figure 5.** Nimodipine inhibits aldosterone-induced ENaC\(\gamma\) expression in vivo. Rats (4 to 8 rats per group) were adrenalectomized and dosed with eplerenone (orally at 10 mg/kg) or nimodipine (orally at 50 mg/kg or via intraperitoneal injection at 20 mg/kg). Aldosterone was injected subcutaneously at 5 μg/kg. The expression of ENaC\(\gamma\) in distal colon was detected using Taqman. *\(P<0.001\) vs aldosterone group, unpaired \(t\) test.

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**Table 2. Potencies of Dihydropyridine CCBs on MR S810L**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μmol/L) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nifedipine</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>14.0 ± 10.4</td>
</tr>
<tr>
<td>Nicardipine</td>
<td>5.10 ± 1.61</td>
</tr>
<tr>
<td>Felodipine</td>
<td>0.44 ± 0.04</td>
</tr>
<tr>
<td>Isradipine</td>
<td>1.06 ± 0.21</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>2.72 ± 0.45</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>0.58 ± 0.10</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>1.37 ± 0.29</td>
</tr>
</tbody>
</table>
Hemorrhage.22,23 MR antagonists have been shown to be cerebral vasospasm and resultant ischemia after subarachnoid without lowering blood pressure and is thus used to prevent ample, nimodipine selectively increases cerebral blood flow in vitro and in vivo. This surprising finding has a number of implications. First, it raises a question as to whether any of the clinical effects of dihydropyridine CCBs are mediated in part by MR. There is clinical evidence that the range of cerebral vascular injury in animal models.24,25 This effect on cerebral blood flow is reduced or absent in patients treated with other dihydropyridine CCBs,26–28 In patients with cerebral ischemia, the doses of nimodipine used produce plasma levels over 200 nmol/L in plasma,29 above the IC50 value for MR. In contrast, commonly achieved plasma levels of the other MR-active dihydropyridine CCBs such as felodipine (peak plasma concentration \( \approx 25 \) nmol/L at 10 mg\( ^{30} \) and nitrendipine (peak plasma concentration \( \approx 50 \) nmol/L at 20 mg\( ^{31} \)) are much lower than their respective IC50 values for MR. The plasma concentration of nifedipine (\( \approx 500 \) nmol/L at 20 mg\( ^{32} \)) can reach to a level close to its IC50 on MR, but nimodipine seems to penetrate blood-brain-barrier more easily than other dihydropyridine CCBs because its brain to plasma ratio is much higher.33 It is thus reasonable to speculate that nimodipine inhibits MR activation in the brain and that this inhibition may also contribute to the beneficial effect of nimodipine on cerebral ischemia and stroke. The main antihypertensive action, however, is still without question via blockade of the L-type calcium channels, where their potency is normally in the 1 to 10 nmol/L range.\(^{13,14} \) In contrast, the lowest MR IC50 is still above 100 nmol/L. Only when high doses are used or if compound accumulates in particular tissues can there be substantial effect on MR. Various formulations have been developed to boost the exposure of CCBs, which could in theory add the benefit of antagonizing MR if such exposure becomes high enough.

Second, our finding offers the probability of novel ligands with good potency at both L-type calcium channels and MR, which might provide better BP control. There are recent patent applications (Takeda Pharm.: WO2005097118; Bayer: DE102005034267) for MR antagonists with the core dihydropyridine structure further suggesting that dihydropyridines could be used as a scaffold for MR antagonists. However, in these applications, no detailed data on either MR activity or CCB activity were shown.

Third, our data suggest that caution should be applied in the interpretation of data from high concentrations of dihydropyridine CCBs. In many in vitro experiments on primary endothelial or smooth muscle cells in which MR is expressed, CCBs have been used at concentrations that could potentially

**Figure 6.** Dihydropyridine can dock into the MR crystal structure. A, Dihydropyridine consensus docking pose in MR LBD illustrated by wild-type and S810L MR LBD crystal structures with Helix 12 truncated to allow for its considerable flexibility.\(^{10} \)\(^{20} \) produced a consensus dihydropyridine binding pose (Figure 6A) with a hydrogen bond from the dihydropyridine ring NH to Asn770, the 4-aryl ring in the A-ring binding pocket, one ester group in the α-face pocket, and the other protruding upward toward the Helix 12 location. In this pose, the dihydropyridine fails to overlap the steroid D-ring region (Figure 6B and 6C) and thus does not make the stabilizing contacts with Helix 11 (including Thr745) thought to be necessary for activation of both wild-type and S810L receptor variants,\(^{10} \) which may underlie its observed antagonistic effect on the S810L mutant.

**Discussion**

We have demonstrated that a number of widely used dihydropyridine CCBs compete for aldosterone binding to MR and function as MR antagonists to block aldosterone-induced coactivator recruitment and transcription of MR target genes in vitro and in vivo. This surprising finding has a number of implications. First, it raises a question as to whether any of the clinical effects of dihydropyridine CCBs are mediated in part by MR. There is clinical evidence that the range of dihydropyridines tested show differential effects.\(^{1,21} \) For example, nimodipine selectively increases cerebral blood flow without lowering blood pressure and is thus used to prevent cerebral vasospasm and resultant ischemia after subarachnoid hemorrhage.\(^{22,23} \) MR antagonists have been shown to be beneficial in increasing cerebral blood flow and reducing cerebral vascular injury in animal models.\(^{24,25} \) This effect on cerebral blood flow is reduced or absent in patients treated with other dihydropyridine CCBs.\(^{26–28} \)
inhibit MR\textsuperscript{34,35}; some effects, particularly those observed only at high concentrations, may be better explained by inhibition of MR.

Fourth, our results provide another example of finding new “homes” for known chemicals. A number of recent reports have found new targets for known drugs, compounds, or active ingredients from herbal medicines.\textsuperscript{36–39} Interestingly, nuclear receptors seem to be the targets most frequently found, presumably reflecting that nuclear receptors evolved to respond to small molecules. A systematic effort to screen known drugs, compounds, and active ingredients from herbal medicines against a full panel of nuclear receptors may thus be fruitful.

Finally, it is interesting to note that, in contrast with eplerenone and spironolactone, the dihydropyridine CCBs tested can fully antagonize the S810L mutant. The docking study suggests that a lack of interaction with Helix 11 might contribute to the loss of agonist activity with the dihyropyridines. Identifying MR antagonists devoid of Helix 11 interaction could thus yield novel compounds that inhibit both wild-type and the S810L mutant. It is also reasonable to speculate that high doses of MR-active dihyropyridines could potentially be a useful therapy for individuals carrying the S810L mutation.

**Perspectives**

The present study demonstrated that a number of marketed dihydropyridine CCBs are potent MR antagonists. This finding suggests that some of the pleiotropic effects of CCBs can be attributed to inhibition of MR. More importantly, it offers an opportunity to identify novel compounds with good potency at both calcium channel and MR, which might provide better BP control and improve compliance as currently most hypertensive patients need multiple drugs to reach BP goal.

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**Disclosures**

J.W.F. is a paid consultant of Pfizer. All other authors are employees of Pfizer and own significant Pfizer stocks.

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