CHAPTER 7

THE EFFECTS OF DIHYDROPYRIDINE AND PHENYLALKYLAMINE CALCIUM ANTAGONIST CLASSES ON AUTONOMIC FUNCTION IN HYPERTENSION: THE VAMPHYRE STUDY

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SUMMARY

The aim of the present study was to compare the effects of a long–acting dihydropyridine (amlodipine) and a nondihydropyridine (verapamil) on autonomic function in patients with mild to moderate hypertension. A total of 145 patients with a diastolic blood pressure (BP) between 95 and 110 mmHg received 8 weeks of verapamil sustained release (240 mg) and amlodipine (5 mg) in a prospective randomized, double blind, cross–over study, both after 4 weeks of placebo. The 24–h autonomic balance was measured by analysis of 24–h heart rate variability and short–term autonomic control of BP by baroreflex sensitivity measurements. Plasma norepinephrine was sampled at rest. Blood pressure was equally reduced from 153/100 mmHg to 139/91 mmHg with verapamil and 138/91 mmHg with amlodipine, \( P=0.50/.59 \). The low–to high–frequency ratio (LF/HF), reflecting sympathovagal balance, was higher with amlodipine than with verapamil (4.66 \( v \) 4.10; \( P=0.001 \)). Baroreflex function was improved by both treatments; however, baroreflex sensitivity (BRS) was significantly higher with verapamil than with amlodipine (8.47 \( v \) 8.06 msec/mmHg; \( P=0.01 \)). Plasma norepinephrine (NE) level was higher with amlodipine than with verapamil (1.59 \( v \) 1.32 nmol/L; \( P<0.0001 \)).

Amlodipine induces a shift in sympathovagal balance, as measured by heart rate variability indices and plasma NE, toward sympathetic predominance compared with vagal predominance with verapamil. Short–term autonomic control of BP, as assessed by BRS, is more effectively improved by verapamil than by amlodipine. These contrasting effects on autonomic function suggest that the nondihydropyridine calcium antagonist verapamil may have additional beneficial effects beyond lowering BP compared with the dihydropyridine amlodipine.

INTRODUCTION

Increased heart rate has long been identified as a marker of sympathetic predominance in hypertension and is related to a higher mortality.\(^1\) Spectral analysis of heart rate variability (HRV) and recordings of muscle sympathetic nerve activity by microneurography have shown increased sympathetic activity and decreased vagal activity in hyper-
tension. Measurement of baroreflex sensitivity (BRS) by cross-spectral analysis and phenylephrine infusions has revealed a diminished baroreflex function in hypertension. Although it is still unclear whether these changes to the autonomic nervous system are pathogenetically involved or are secondary to consequences of chronically elevated blood pressure (BP), they may contribute to the increased cardiovascular risk in established hypertension. Reducing sympathetic tone and restoration of baroreflex function by antihypertensive drugs could therefore be beneficial; or, at least, antihypertensive drugs should not deteriorate them.

Calcium antagonists are widely used to treat hypertension. However, the influences on the autonomic nervous system may differ between the various classes. The use of short-acting calcium antagonists has been associated with increased morbidity and mortality in patients with hypertension and coronary artery disease. It has been suggested that this could at least partly be due to reflex sympathetic activation in response to vasodilation and a reduction of peripheral resistance. In contrast to short-acting dihydropyridines (DHP), long-acting DHP and non-DHP would have similar effects and would not increase sympathetic activity. However, very few data are available from direct comparisons of the effects of the latter two classes on autonomic function in hypertension. Therefore, the aim of the present study was to compare the effects of a long-acting dihydropyridine (amlodipine) and a nondihydropyridine (verapamil) on autonomic function in patients with mild to moderate hypertension in a multinational prospective, double-blind, randomized, cross-over comparison. The low-to-high-frequency (LF/HF) ratio and BRS were chosen as primary efficacy parameters to evaluate the effects of the drugs on 24-h sympathovagal balance and on short-term reflex autonomic control of BP.

**METHODS**

**Study Design and Subjects.** The effects of Verapamil and Amlodipine on autonomic function in Patients with Hypertension at Rest and during Exercise (VAMPHYRE) study was a comparison of verapamil sustained release (SR, 240 mg) and amlodipine (5 mg) in hypertensive patients >18 years of age. Hypertension was defined as a diastolic BP...
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(DBP) ≥95 mmHg on at least three occasions. In these patients, either hypertension was newly diagnosed or the current antihypertensive treatment did not meet therapeutic goals (systolic BP [SBP] <140 mmHg and DBP 90 <mmHg). Patients were excluded if secondary hypertension was suspected or if they had had a recent cardiovascular event, had diabetes mellitus, or used drugs known to influence the autonomic nervous system. Seven centers in six European Community countries participated. Informed consent was obtained from all patients. The local ethics committees at each participating hospital approved the VAMPHYRE protocol.

At the first patient visit, any antihypertensive treatment was stopped and 4 weeks of placebo treatment was started. Sitting BP was measured three times after 10 min of rest with 2–min intervals. Randomization to sequence 1 (8 weeks of verapamil, 4 weeks of placebo, 8 weeks of amlodipine) or sequence 2 (8 weeks of amlodipine, 4 weeks of placebo, 8 weeks of verapamil) followed at the second visit if DBP was ≥95 and ≤110 mmHg and SBP was ≤180 mmHg. The first placebo period was open; both active drug periods were double–blind; and the second placebo period was single–blind. Blood pressure measurement and all assessments of autonomic function were performed at the start of the study and after each placebo and each treatment period.

**Study Procedure.** All tests were performed in the morning in a warm, quiet room. Patients refrained from eating and smoking and from drinking alcohol, coffee, and tea that day. A Finapres (Ohmeda 2300; Ohmeda, Liberty Corner, NJ) blood pressure monitor was attached to the third finger of the patient’s right hand. Ten min after insertion of an intravenous catheter, heart rate and sitting and supine BP were measured. Venous blood, 6 mL, was sampled in a prechilled EGTA–glutathione tube for determination of plasma norepinephrine (NE) level by electrochemical detection after high–pressure liquid chromatography in a central laboratory (Analytico, Breda, the Netherlands) for all centers. After 30 min of rest, BRS was measured; then a 24–h Holter electrocardiographic (ECG) recorder was applied. Because the autonomic nervous system is very much influenced by mental stress, the purpose of the first visit was only to get the patient acquainted with the study procedures. No ECG recorder was applied on the first visit.
Holter ECG: Heart Rate Variability Analysis. Twenty-four-hour ambulatory ECG recordings were acquired by a Marquette Holter recorder (Series 8500) (Marquette Electronics Inc., Milwaukee, WI) and analyzed by an experienced analyst. Three ECG leads (modified leads V1, V5, and a VF) and a time signal to correct for tape speed irregularities were recorded. The HRV was analyzed with the COHORT program, as described previously\textsuperscript{16} and in accordance with the recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.\textsuperscript{17} Low-frequency power (LF, 0.04 to 0.15 Hz) reflects sympathetic and partly vagal activity in contrast to high-frequency power (HF, 0.15 to 0.40 Hz), which primarily reflects vagal activity.\textsuperscript{18} The LF/HF ratio is the ratio of LF and HF powers and is considered to reflect sympathovagal balance.\textsuperscript{13,14}

Baroreflex Sensitivity Measurement. Three hundred-second segments of Finapres recordings during rest were used to determine the BRS with the CARSPAN program (IEC ProGamma, the Netherlands), as described previously.\textsuperscript{15} After detection and correction of artifacts, BRS was calculated by the transfer function method, defining BRS as the mean modulus of the cross-spectrum of SBP and R–peak-to–R–peak (RR) interval variability in the 0.07–to 0.15–Hz frequency band with at least 0.5 coherence. The phase between SBP and RR interval length spectra indicates the delay between a BP variation and the subsequent adaptation of RR interval length. Because RR interval modulation usually follows a change in BP, the phase is mostly a negative value. As vagal reflexes act more rapidly than sympathetic reflexes, a change in phase toward a less negative value (ie, a shorter delay from change in BP to RR interval response) indicates a shift toward vagal predominance. In contrast, a change in phase toward a more negative value (ie, a longer delay) indicates a shift toward sympathetic predominance.\textsuperscript{19}

Sample Size and Statistical Analysis. Monitoring and statistical analysis (SAS software 6.12; SAS Institute, Cary, NC) were performed by an independent monitoring agency (TCC, Groningen, the Netherlands). Sample-size calculation was based on the 24–h LF/HF ratio of a recent trial performed in our hospital.\textsuperscript{16} Anticipating a standard deviation of 2.5, a total of 130 evaluable patients (65 in each sequence) were needed to detect a difference of 0.875 in a two-sided \( t \) test for \( \alpha=0.05 \) with 80% power.
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Primary statistical analysis was performed on the intention-to-treat population, which consisted of all patients randomized with at least one dose of study medication taken. The per protocol population was defined as all patients with all four fully evaluable Holter ECG and no major protocol violations. A mixed analysis of covariance model was defined to test the null hypothesis of no difference between both treatments. Period, treatment, and center were defined as fixed factors, the placebo period preceding the active drug as covariate, and patient within center as random effect to account for the intrapatient correlation in this cross-over setting. A $P$ value $<0.05$ in the two-sided test was considered statistically significant. The student $t$ test was performed for all endpoints to check for any differences between the first and second placebo period. All parameters that were nonnormally distributed and could not be transformed to a normal distribution were tested with a Wilcoxon test. Secondary models were defined to exclude the influence of a carry-over effect.

**Results**

**Patient Recruitment, Follow-Up, and Adverse Events.** Of 168 patients who entered the placebo run-in phase, 23 patients left the study because they did not meet randomization criteria, and 145 were randomized at the second visit. Patient characteristics at randomization are summarized in Table 1. Because no significant difference between both placebo periods existed for BP or any autonomic function parameter, the mean

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (Range) or Number (%)</th>
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<tbody>
<tr>
<td>Male/female, $N$ (%)</td>
<td>95/50 (65%/35%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>51 (20–72)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>153 (124–180)</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>100 (95–110)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>67.8 (51–102)</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>28.6 (19.9–46.8)</td>
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<tr>
<td>Smokers, current, $N$ (%)</td>
<td>33 (23%)</td>
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<tr>
<td>Dyslipidemia, $N$ (%)*</td>
<td>57 (40%)</td>
</tr>
<tr>
<td>Family history of premature atherosclerotic disease, $N$ (%)</td>
<td>13 (9%)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>85 (59–141)</td>
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* Dyslipidemia is defined as total cholesterol $>6.5$ mmol/L, low-density lipoprotein $>5$ mmol/L, high-density lipoprotein $<0.9$ mmol/L, or triglycerides $>2.5$ mmol/L.
of both is shown. A total of 22 patients later dropped out of the study: 13 during verapamil use, four during the second placebo period, and five during amlodipine use. The mean compliance, assessed by tablet count, was >95% for all treatments. The main reasons for premature study discontinuation were adverse events and lack of compliance. The design of the study does not allow an unbiased assessment of the relation between adverse events and study drugs. The adverse events with a probable relation with the drugs were most frequently common side effects: headache, dizziness, palpitations, constipation, and peripheral edema. However, three generalized allergic responses to verapamil that faded after withdrawal of the drug were observed.

**Blood Pressure and Heart Rate Response.** After placebo, the mean SBP and DBP were 153 and 100 mmHg, respectively. Both were equal after verapamil and amlodipine treatment (139/91 vs 138/91 mmHg; P = .50/.59). After placebo, mean heart rate was 68 beats/min. Heart rate was lower with verapamil than with amlodipine (65 vs 69 beats/min; P < .0001).

**Twenty-Four-Hour Heart Rate Variability.** Of the 528 Holter ECG that were applied, 453 had at least 18 h suitable for HRV analysis after removal of artifacts, nonstationary segments, and segments with >15% ectopy (Figure 1). After placebo, the primary endpoint LF/HF ratio was 4.54. The LF/HF ratio was higher after amlodipine than after verapamil (4.66 vs 4.10). This difference was highly significant in both in the intention-to-treat (P = 0.001) and per-protocol (P = 0.01) analyses. No change in overall HRV (total power) were observed after treatment.

**Baroreflex Sensitivity.** Both verapamil (+27%) and amlodipine (+21%)
increased BRS (Table 2), but BRS was significantly higher after verapamil ($P=0.01$). Short-term BPV was reduced by both verapamil (~45%) and amlodipine (~30%), but was significantly lower with verapamil ($P<.0001$). Phase was significantly shorter during verapamil treatment ($P<.0001$).

**Plasma Norepinephrine.** Of the 477 samples drawn, two were below detection limit (<0.10 nmol/L). After placebo, plasma NE level was 1.35 nmol/L. Verapamil did not change plasma NE, but amlodipine increased plasma NE by 22%, resulting in significantly higher NE levels compared with those achieved with verapamil (1.59 vs 1.32 nmol/L; $P<.0001$, Figure 2).
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**DISCUSSION**

This study demonstrates that the effects of the long–acting dihydropyridine amlodipine and the phenylalkylamine verapamil on the autonomic nervous system are markedly different, whereas the reduction in BP by the two drugs is similar.

**Effects on Sympathovagal Balance.** The primary endpoint, LF/HF ratio, was significantly higher with amlodipine compared with verapamil, suggesting sympathetic predominance during amlodipine treatment compared with vagal predominance during verapamil treatment. The other indices of sympathovagal interaction, heart rate, and phase between short–term BP and HRV showed the same contrasting effects. One might object that the contrasting effect on LF/HF ratio is due to the heart rate–lowering effect of verapamil (caused by the direct effect of verapamil on the sinus node), rather than to modulation of the sympathovagal balance. However, when we added the mean 24–h RR interval length to the model as a covariate to study the influence of the effects of heart rate on the primary outcome, the difference in LF/HF ratio between verapamil and amlodipine remained significant (4.20 vs 4.55, \( P=0.03 \)). These contrasting effects on sympathovagal balance, as measured by LF/HF ratio, could result from modulation of sympathetic or vagal activity, or both. Compared with baseline level, amlodipine

![Figure 2. Plasma norepinephrine before and after treatment with verapamil and amlodipine. Data given as mean (SEM). *P<0.001 verapamil vs amlodipine.](image)
increased plasma NE, whereas verapamil had no effect. The HF power (reflecting vagal modulation) increased with verapamil, in contrast to the absence of effect with amlodipine. These findings imply that amlo
dipine increased sympathetic activity, whereas verapamil increased 
vagal tone.

There is no consensus whether amlodipine raises sympathovagal bal-
ance in hypertension when given long term. De Champlain et al found 
that amlodipine raised heart rate and increased plasma NE by 50% 
after 6 weeks therapy. In contrast, Hamada et al reported no changes 
in heart rate or urinary NE and decreases in plasma NE and LF/HF 
ratio after 4 weeks of amlodipine in a comparison with nifedipine and 
slow-release nifedipine. However, large differences in baseline values 
of urinary and plasma NE and of heart rate existed between the small 
groups (n=16), which often exceeded the treatment effect. Sakata et al 
found that amlodipine did not significantly change plasma NE in 24 
patients after 2 weeks to 3 months, although the absolute difference 
between mean plasma NE before and after therapy was about as large 
(increase of 0.24 nmol/L) as in our study. However, all of these studies 
had a relatively small sample size (n=16 to 24 in the amlodipine group) 
and a parallel design, therefore increasing the chance of a type II error. 
In contrast, the 145 patients and the cross-over design of the present 
study result in a considerably greater statistical power.

The heart rate–moderating effect of verapamil is well documented. Furthermore, verapamil is thought either to not affect plasma NE or to decrease it. In one of the few studies with a cross-over design, 4 weeks of felodipine had no effect on heart rate and plasma NE, whereas 4 weeks of verapamil lowered both. In a review of 20 clinical studies 
reporting the long-term effects of long-acting calcium antagonists in 
patients with hypertension, heart rate was unchanged; plasma NE lev-
els increased by 14.5% ± 5% after long-acting DHP calcium antagonists; 
and heart rate and plasma NE levels decreased after long-acting non- 
DHP calcium antagonists by 7.1% ± 1.8% and 20.7% ± 9.8%, respectively 
(P<0.001 between groups for both comparisons).

Our study supports the notion of opposite effects of verapamil and 
amlo
dipine on sympathovagal balance, as assessed by HRV indices and 
plasma NE. Vasodilation induced by amlodipine probably accounts for 
the reflex increase of sympathetic activity. Nazzaro et al showed, be-
sides a decrease in heart rate with verapamil and an increase in heart
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rate with amlodipine, that both drugs reduced BP and total vascular resistance equally in 23 hypertensive patients.1 Possibly, reflex increase of sympathetic activity is counteracted by a direct inhibition of presynaptic release of NE by verapamil.2,3 Alternatively, verapamil might directly decrease sympathetic activity or increase vagal activity by a central effect on the cardiovascular control center, inasmuch as verapamil can pass the blood–brain barrier.4

The changes in heart rate, LF/HF ratio, and plasma NE were negatively associated with their baseline levels for both amlodipine (Spearman's $r=-0.34, P=0.004; r=-0.38, P=0.004; r=-0.23, P=0.07$, respectively) and verapamil ($r=-0.58, P=0.001; r=-0.25, P=0.10; r=-0.47, P=0.0006$, respectively). As all these parameters tended to increase with amlodipine and to decrease with verapamil, one might suggest that subjects with a relatively low sympathetic activity before treatment show the largest increase of sympathetic activity with amlodipine. In contrast, subjects with a relatively high sympathetic activity before treatment might benefit most from treatment with verapamil in terms of a reduction of sympathetic activity, as assessed by heart rate and plasma NE.

**Effects on Short–Term Autonomic Control of Blood Pressure.** Although both drugs improved BRS, this effect was considerably greater with verapamil than with amlodipine. Increased autonomic control of heart rate, as a consequence of increased vagal tone during verapamil treatment, might explain this. Kailasam et al reported no significant differences among 2 weeks of placebo, 4 weeks of felodipine, and 4 weeks of verapamil on phenylephrine BRS, although BRS was higher during verapamil treatment (9.3 msec/mmHg) compared with felodipine (5.9 msec/mmHg).5 However, sample size was small ($n=15$). Second, the different BRS technique could explain the discrepancy with our results. Although the transfer function technique (present study) and the phenylephrine technique (Kailasam et al) are highly correlated,5,15 the phenylephrine technique has been criticized because the drug influences the baroreflex itself,6 whereas the transfer function technique analyzes the baroreflex response to spontaneous variations in BP.6

**Clinical Implications and Limitations of the Study.** Our study demonstrates that the effects of different classes of calcium antagonists on sympathovagal balance and baroreflex function, as measured by HRV,
BRS, and plasma NE, are markedly different in hypertension. However, a number of issues have to be considered before clinical implications can be drawn.

First, there is debate about how to measure sympathovagal balance and whether noninvasive HRV methods are suitable. However, an increase in LF/HF ratio observed during tilt that can be attenuated by β-blockade suggests that an increase in LF/HF ratio reflects a shift toward sympathetic predominance. Furthermore, the contrasting effects of verapamil and amlodipine on LF/HF are endorsed by their similar effects on heart rate, phase between BP and heart rate variability, and plasma NE. Second, the present study is a short-term study with a treatment duration of two times 8 weeks. Although this period is probably long enough to overcome short-term effects and to have a stable autonomic balance, the long-term effects have to be established in studies with longer duration.

Still, the present study may have clinical implications. Because impaired baroreflex function and increased sympathovagal balance often accompany hypertension, recuperation of baroreflex function and autonomic balance may be valuable additional goals in the treatment of hypertension. Unfortunately, no trial has yet been designed to show that reduction of sympathovagal balance reduces cardiovascular mortality in hypertension. However, there is considerable circumstantial evidence. First, increased heart rate is a marker of increased risk for death from coronary heart disease, cardiovascular disease, and even for all causes of mortality in hypertension. Although the predictive value of BRS and HRV has not yet been proved in hypertension, these parameters are markers of increased risk after myocardial infarction and in the general population. Second, several pathophysiologic mechanisms may account for this increased risk. Chronically increased sympathovagal balance is not only associated with a higher BP and heart rate, but also with end-organ damage (left ventricular hypertrophy), increased platelet coagulation, increased plasma cholesterol and triglycerides, increased plasma glucose, increased insulin, and increased hematocrit—and arrhythmias. Third, a reduction in end-organ damage (assessed by left ventricular hypertrophy), which was confined to hypertensive patients with a reduction in sympathetic tone, supports their close association.
CONCLUSION

We conclude that 1) the long-acting dihydropyridine amlodipine induces a shift in sympathovagal balance, as assessed by HRV and plasma NE, toward sympathetic predominance compared with vagal predominance with the non-dihydropyridine verapamil; and 2) short-term autonomic control of BP, as measured by BRS, is more effectively restored by verapamil than by amlodipine. These contrasting effects of calcium antagonists of different classes may have therapeutic implications beyond lowering BP. However, further long-term studies are needed to determine whether reduction of sympathetic tone and restoration of baroreflex function can reduce the risk of complications and mortality in hypertension.
REFERENCES


