Autonomic dysfunction in cardiovascular disease
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Chapter 9

Less Adrenergic Response to Mental Task During Verapamil Compared to Amlodipine Treatment in Hypertensive Subjects


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**SUMMARY**

We compared the effects of amlodipine and verapamil slow release on autonomic responses to a 5–min mental arithmetic test (MST) in patients with mild to moderate hypertension. Twenty subjects received 8 weeks of verapamil slow release 240 mg or amlodipine 10 mg in a double–blind crossover design, both after 4 weeks’ placebo. Heart rate (HR) and blood pressure (BP) were continuously monitored. Venous plasma catecholamines were analysed by a radioenzymatic assay. Baroreflex sensitivity (BRS) was estimated with the transfer function technique. Calculations of the area under the curve (AUC) were used to estimate average HR, BP and catecholamine concentrations. The reactivity to MST was estimated as percent change from the basal AUC. A paired t–test was performed. Data are means ± SEM. Compared to verapamil, amlodipine increased average noradrenaline (NA) concentrations (245 ± 23 vs 191 ± 17 pg/l, respectively, p=0.005), NA reactivity (14.0 ± 5.5% vs −2.9 ± 3.3, p=0.004), average HR (65 ± 2 vs 61 ± 2 beats/min, p<0.001) and HR reactivity (2.5 ± 1.0 vs 0.1 ± 0.9%, p=0.056). BP did not differ significantly. BRS correlated with average and baseline HR on both medications (r=−0.53 and −0.63, p≤0.03). We conclude that adrenergic responses to MST are blunted on treatment with verapamil compared to amlodipine in hypertensive patients.

**INTRODUCTION**

Surges of adrenergic activity as seen during mental stress trigger cardiac events and ventricular arrhythmias1–3. A major goal in treatment of patients with coronary artery disease (CAD) is to prevent such events4. Calcium channel blockers are used in treatment of CAD, but preferably in those who are not tolerating adrenergic betablockers4,5. Both drugs are widely used in treatment of hypertension5.

Calcium–channel blockers are a heterogeneous group with diverging effects on the autonomic nervous system. Grossman et al.6 meta–analysed studies which involved 1252 hypertensive patients treated with various calcium antagonists. After more than 1 week’s medication, heart rate (HR) was unchanged and noradrenaline (NA) increased (15%) on treatment with long acting dihydropyridines (e.g. amlodipine,
nisoldipine), while the nondihydropyridines (e.g. verapamil slow release (SR), diltiazem) reduced HR (7%) and NA (21%).

Gebara et al.\(^7\) showed lower overall systolic blood pressure (SBP) and blood platelet aggregability during mental stress (MST) and cold pressor test (CPT) on verapamil SR than placebo medication in 13 moderately hypertensive patients. Ludwig et al.\(^8\) demonstrated higher concentrations of NA, but no HR differences and lower blood pressure (BP) during MST on treatment with nisoldipine compared to placebo in moderately hypertensive subjects. Nazzaro et al.\(^9\) demonstrated reduced overall HR but similar BP response to the total load of a battery of stress tests (two different MSTs, CPT and isometric handgrip) on verapamil SR treatment compared to amlodipine in 23 moderately hypertensive patients. However, these tests trigger different stress responses\(^10\)–\(^14\).

Thus, by not differentiating between the different tests, important information might have been lost. To our knowledge, nobody has addressed changes in cardiovascular and plasma catecholamine reactivity during specific mental stress on dihydropyridine compared to verapamil treatment. Thus, in the present study we wanted to compare the effects of amlodipine and verapamil SR on autonomic responses to MST in patients with mild to moderate hypertension. Both drugs are widely used in treatment of CAD and hypertension. MST was preferred, as this test evokes the “fight and flight” response\(^10,13,14\), which closely resembles an “everyday situation” that may trigger cardiac events. Furthermore, as baroreceptor reflex sensitivity (BRS) is reduced in hypertensive patients\(^15\), and decreases during mental stress\(^16\) we wanted to relate BRS to the autonomic responses during MST.

**Materials and Methods**

The present study was a substudy to the Vamphyre study, “The effects on autonomic function of Verapamil versus Amlodipine in Patients with mild to moderate Hypertension during Rest and Exercise” which was carried out in six European countries. Patients’ entry were eligible if they were >18 years old, and suffered from mild to moderate hypertension [140≤systolic blood pressure (SBP)≤180, diastolic blood pressure (DBP) ≥90 mmHg]. Patients were excluded if secondary hypertension
was suspected, if they had recently suffered a cardiovascular event, if organ failure was present or they had diabetes mellitus, autoimmune disease or Parkinson’s Disease. At the first visit, hypertensive treatment, if any, was stopped and 4 weeks’ placebo treatment was started. Randomization to sequence 1 (8 weeks verapamil, 4 weeks placebo, 8 weeks amlodipine) or 2 (8 weeks amlodipine, 4 weeks placebo, 8 weeks verapamil) followed at the second visit if DBP was ≥95 and μ110 mmHg and SBP μ180 mmHg. The patients were scheduled for five visits. Visit 1 took place the first day of the study, the remaining visits were performed at the last day of each medication period. The first placebo period was open, the second placebo period was single blind and both active drug periods were double blind. The purpose of visit one was to make the patient acquainted to the study procedures. The patients who terminated antihypertensive medication at visit 1 were scheduled for a visit to check BP 1 week later.

Patients were recruited from the outpatient clinic for hypertensive patients, Ullevål University Hospital, Oslo, Norway. The Regional Board of Research Ethics approved the study, and informed written consent was obtained from each participant.

Subjects. Twenty–three patients entered the study between March 1997 and February 1998. Two patients did not want to perform the MST. One patient was withdrawn from the study due to an eye vein thrombosis after the first medication period. The remaining 20 patients stayed throughout the study, and consisted of 10 male and 10 female participants. Before visit 1, 11 patients did not use any vasoactive drug, six used a calcium antagonist, two used an ACE inhibitor and one used a beta–blocker. Three participants were smokers. Mean age was 55 years (range 34–70 years). Mean body mass index was 26.2 ± 0.6 kg/m2. Mean sitting BP and HR were 151 ± 3/99 ± 1 mmHg and 71 ± 2 beats/min, respectively, at the second visit. Data are means ± SEM.

The medication compliance, assessed by tablet count, was better than 80% for all drugs.

Study procedure. All examinations were performed in the morning in a quiet room with temperature of 22–24°C, after an overnight fast and a refrain from alcohol and tobacco the last 24 h. HR and sitting sphygmomanometric SBP and DBP (Korotkoff sounds I and V) were
measured three times after a 10-min rest with a 2-min interval. The final BP was averaged from these three measurements. Beat–to–beat BP and HR were recorded in the supine position with a Finapres (Finapres, Ohmeda 2300, Englewood, Colorado, USA) non–invasive blood pressure monitor with the appropriate cuff applied to the third finger of the left hand. This instrument has been validated, and the accuracy and precision found sufficient for tracking of changes in BP and HR17.

Mental stress test. The subjects were asked to subtract a two–digit number, starting with a four–digit number for 5 min as fast as possible. All wrong calculations were corrected. A metronome making noise at a frequency of 2 Hz was used to distract the subjects. The numbers used for subtractions were changed within the different examinations. The subjects rested for 10 min in the supine position before and after finishing MST. MST was carried out 13–14 h after the last dose of study medication.

The Finapres software automatically registered HR, SBP and DBP 1 min before starting the subtractions, and after 1, 3 and 5 min calculation and 2, 5, 7 and 10 min after finishing the subtractions.

Mean arterial pressure (MAP) was calculated with the formula: MAP=DBP + 1/3 (SBP–DBP).

Baroreceptor reflex sensitivity. Finapres recordings of 300–s segments beat–to–beat BP and HR during rest in the supine position and during the 5–min stress were used for determination of the BRS with the CARS-PAN program (ProGAMMA bv, Groningen, Netherlands), as described previously15,16. This program allows discrete Fourier transformation of nonequidistant samples of BP and RR–interval series. Nonstationary signals or periods with less than 90% normal–to–normal beats were excluded. Subsequently, spectral analysis of SBP and RR–interval length was performed, and BRS calculated with the “Transfer Function” method. This method defines the BRS as the mean modulus between SBP and RR–interval length spectra in the midfrequency band (0.07–0.14 Hz) with a coherence of more than 0.5. BRS is expressed in ms/mmHg.

Catecholamines. Venous blood was sampled through an 18–gauge Venflon (BOC, Ohmeda AB, Helsingborg, Sweden) in a cubital vein 1 min before starting the subtractions, after 1 and 5 min calculations and 10 min after finishing subtractions. Blood was sampled on prechilled
EGTA–glutathion tubes, then centrifuged and the plasmas frozen at –70°C. Plasma catecholamines were analysed by a sensitive radioenzymatic technique\textsuperscript{18}.

**Data analysis and statistics.** The data were analysed using SPSS 9.0.1 statistical package (SPSS Inc, Chicago, IL, USA). Non–normally distributed data were natural log transformed. Two–tailed statistical analyses of data were carried out using Student’s \textit{t}–test for paired samples (\(p\)), Spearman’s correlation coefficient was also used. The HR, MAP and catecholamine reactivity to MST were estimated as percentage change in the area–under–the–curve (AUC) from baseline AUC as described previously\textsuperscript{9,19}. Baseline AUC was defined as baseline value multiplied with the total duration of the monitored period (16 min). Average levels were defined as the AUC for the measured parameters divided by the length of the monitored period. The level of statistical significance was set at \(p=0.05\). Data are presented as means ± SEM. The study was designed to compare results from the examinations after the two periods with amlodipine and verapamil.

**RESULTS**

**Catecholamines.** Average plasma NA was lower on verapamil than amlodipine treatment, 191 ± 17 pg/l vs 245 ± 23 pg/l, respectively, \(p=0.005\). NA reactivity was abolished on verapamil while it was present on amlodipine medication, −2.9 ± 3.3% vs 14.0 ± 5.5%, respectively, \(p=0.004\) (Figure 1). Average plasma A on verapamil was 26 ± 4 pg/l vs 32 ± 5 pg/l on amlodipine, \(p=0.5\). The A reactivity on verapamil was 25.7 ± 11.2% vs 34.3 ± 13.5% on amlodipine, \(p=0.9\) (Figure 1).

**Heart rate and blood pressure.** Average HR was lower on verapamil than amlodipine treatment, 61 ± 2 beats/min vs 65 ± 2 beats/min, \(p<0.001\). HR reactivity was lower on verapamil compared to amlodipine medication, 0.1 ± 0.9% vs 2.5 ± 1.0%, respectively, \(p=0.056\) (not significant; Figure 1).

BP reactivity, overall and baseline BP did not differ significantly between the two medication periods (Figure 1). Baseline BP on verapamil and amlodipine treatment were 146 ± 3/93 ± 2 mmHg and 144 ± 2/91 ±
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Baroreceptor reflex sensitivity. BRS was calculated by two independent investigators. The two BRS estimations correlated significantly \((r=0.96, p<0.001)\).

BRS estimated during the 5–min mental stress correlated with average HR, i.e. the total number of heartbeats spent during the monitored period on verapamil and amlodipine medication, \(r=-0.63\) and \(-0.53\), respectively, the regression coefficient \((\beta)\) was \(-18.9\) and \(-22.2\), respectively, \(p\leq0.05\) (Figure 2). Baseline HR also correlated, \(r=-0.53\) and \(-0.49\), respectively; \(\beta\) was \(-1.0\) and \(-1.4\), respectively, \(p\leq0.03\).

We did not find any significant BRS differences between the two medication periods, although there was a tendency of higher BRS both at rest and during MST on verapamil compared to amlodipine \((p>0.2)\).

**DISCUSSION**

The present study demonstrated that verapamil, when compared to amlodipine treatment, reduced HR and NA reactivity to MST, as

Figure 1. Plasma noradrenaline (NA), mean arterial pressure (MAP), heart rate (HR) and plasma adrenaline (A) changes during mental stress test (MST) in 20 hypertensive subjects. Data are means ± SEM. Plasma NA was lower on verapamil treatment compared to amlodipine treatment after 1 and 5 min MST, and 10 min after finishing the calculations, \(p\leq0.001\). The NA reactivity was blunted on verapamil compared to amlodipine treatment, \(p=0.006\). There was no significant difference in MAP between the two medication periods. Overall HR was lower and the HR reactivity was lower on verapamil compared to amlodipine treatment. There was no significant difference in A between the two medication periods. Amlodipine – – – –, Verapamil — — —.
well as average HR and NA in hypertensive patients. BRS estimated during the 5–min MST correlated with the total number of heartbeats throughout the monitored period, as well as baseline and average HR. Both medications lowered BP effectively and to a similar extent.

Our main and novel finding is a substantial reduction of sympathetic reactivity to mental stress on verapamil treatment compared to amlodipine treatment.

Our findings during rest are in accordance with previous studies regarding NA, BP and HR. The cardiovascular responses to mental stress are in accordance with the observations by Nazzaro et al. although they observed the responses to the total load of a battery of tests.

Dihydropyridines lower BP mainly through dilation of peripheral resistance vessels. The drop in BP is sensed by the arterial baroreceptors, which through a reflex arc involving the cardiovascular centre in the brain stem increases the sympathetic activity. Verapamil is a less potent vasodilator, but has in addition depressant effect on the atrioventricular conduction and negative cardiac inotropic effect. These unlike pharmacological properties may partly explain the less sympathetic triggering on verapamil compared to amlodipine medication. Increased sympathetic activity decreases BRS. We demon-

Figure 2. Baroreceptor reflex sensitivity (BRS) vs total number of heartbeats during mental stress test (MST) in 20 hypertensive patients. BRS correlated with the total number of heartbeats both on verapamil and amlodipine medication, r = –0.63 and –0.53, respectively, the regression coefficient was –18.9 and –22.2, respectively, p ≤ 0.05 and p ≤ 0.02. Verapamil regression line ---; amlodipine regression line --; verapamil (●) and amlodipine (○).
strated a negative correlation between the counted heart beats during the test and BRS, i.e. the higher the number of heart beats, the lower the BRS. Thus, as BRS decreases, the ability to buffer blood pressure alterations by changing the HR goes down.

There may also be an alternative explanation of our finding. Terland et al.\textsuperscript{22} have demonstrated that both dihydropyridine and non–dihydropyridine calcium antagonists reduce the uptake of NA in adrenomedullary catecholamine storage vesicles due to inhibition of the H+–ATPase, which preserves an acidic interior of the vesicles. Moreover, amlodipine carries an amino group, which is protonized in the interior of the granula\textsuperscript{22}. Influx of amlodipine into the storage vesicles is therefore followed by alkalization\textsuperscript{22}, which also may contribute to the inhibition of NA uptake, especially on treatment with amlodipine. These mechanisms may thus reduce the local clearance of NA in the synaptic cleft, and thereby contribute to increased stimulation of adrenergic receptors.

The baseline HR and plasma NA tended to be higher than the recovery measurements, probably due to the announcement effect\textsuperscript{10}. However, as delta basal-recovery did not deviate significantly between the two medication periods, it is unlikely that different announcement effects justify the reactivity differences. On the contrary, this effect might explain the negative reactivity in some subjects.

Calcium antagonists are widely used in treatment of hypertension, which is a well–known risk factor for cardiovascular disease\textsuperscript{23}. They also represent an alternative drug in treatment of patients with established coronary artery disease, who cannot use beta–blockers\textsuperscript{4}. Surges of sympathetic activity, are known to trigger cardiovascular events\textsuperscript{1,24,25}. Our study has demonstrated a reduced triggering of sympathetic activity to an “everyday” stress situation in hypertensive patients on verapamil treatment compared to amlodipine.

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