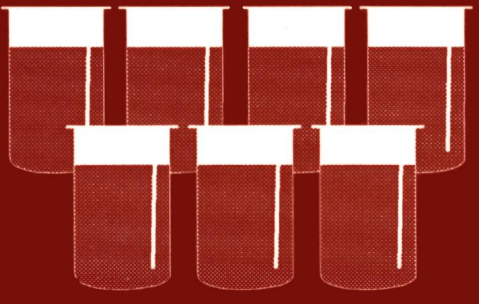
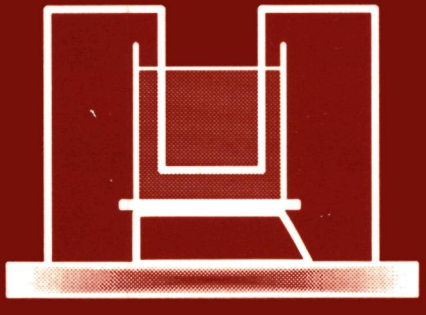
Natriuretic Effects of Dihydropyridine Calcium Entry Blockers





Henk van Hamersvelt

Natriuretic Effects of Dihydropyridine Calcium Entry Blockers.

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de Medische Wetenschappen

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Hendrik Willem van Hamersvelt
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Promotor : Prof. dr. R.A.P. Koene

Co-promotores : Dr. F.Th.M. Huysmans

Dr. J.F.M. Wetzels

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voor Els, Hanneke,
Robbert en Eveline
aan mijn ouders

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CHAPTER 1

Introduction

Background

Systemic hypertension is one of the most common pathological conditions in Westernized societies with an estimated prevalence of up to 25 %[1]. The individual risk of morbidity and mortality associated with hypertension increases progressively with higher blood pressure levels, but the main burden of this illness arises from the masses of people with only minimally elevated blood pressure[2]. Reduction of the incidence of the sequelae of hypertension in individuals will depend mainly on drug treatment, because non-pharmacologic interventions appear to be considerably less effective in lowering blood pressure[3]. Over the past 30 years, protective effects of antihypertensive drugs have been demonstrated for ever lower diastolic blood pressures, the latest trials using 90 mmHg as the threshold for inclusion[4,5]. A growing part of the population thus seems to be eligible for antihypertensive treatment, although the individual benefit will be lower at lower blood pressure levels. The many possible disadvantages of treatment and even of the diagnosis hypertension could thus outweigh the benefits. Kaplan therefore broadened a statement of Rose[6] for a conceptual definition of hypertension[7]: "that level of blood pressure at which the benefits (minus the risks and costs) of action exceed the risks and costs (minus the benefits) of inaction". Thus, the more we reduce the disadvantages of treatment, the larger the proportion of the population which can have benefit from it. A better understanding of the mode of action of specific drugs could lead to more rational choices in individual patients and hopefully a better control of side effects. In this thesis, we have studied two intriguing and at first sight opposite effects of calcium entry blockers, namely their natriuretic action and their propensity to cause edema formation.

General aspects of calcium entry blockers

Calcium entry blockers are a chemically heterogeneous class of drugs which share the ability to inhibit calcium influx into vascular smooth muscle cells, myocardial contractile cells, and conducting cells of the heart[8]. In vitro, the effects of these agents could be reversed by calcium ions, which led Fleckenstein to propose the term calcium antagonists[9]. Although this term is still widely used, the denomination calcium entry blockers (CEB) seems more appropriate. Intracellular free calcium plays an essential role in many cell

functions including muscle contraction. Its concentration can be increased by release from intracellular stores, in particular the sarcoplasmatic reticulum, and by calcium influx through special calcium channels in the plasma membrane which itself is almost impermeable for calcium ions[8]. Calcium channels can be opened either by depolarisation (voltage operated calcium channels) or by hormones such as norepinephrine and angiotensin II (receptor operated calcium channels). These calcium channels play an important physiologic role in excitation-contraction coupling processes in vascular smooth muscle cells and hence regulate vascular tone and peripheral resistance. CEB block the voltage operated channels and thereby induce vasodilation and blood pressure reduction[10]. The voltage operated calcium channel is a protein structure which transverses the lipid bilayer of the plasma membrane. It has been isolated from skeletal muscle and appears to be composed of four subunits ($\alpha 1$,$\alpha 2/\delta$, β, γ)[11]. The different classes of CEB bind to specific receptor domains of the $\alpha 1$-subunit, a 165 kDa protein, which by itself is capable of calcium conduction[11]. However, both binding of CEB and conduction of calcium are greatly influenced by the other three subunits. Calcium channels in heart and vascular smooth muscle cells are less accessible than in skeletal muscle, but $\alpha 1$ and $\alpha 2$ subunits have been identified in these tissues. Very recently, also β subunits were demonstrated in the human heart[12]. Molecular cloning techniques have revealed a great diversity among calcium channels, arising from multiple genes and alternative splicing in gene products[13]. Thus, with the anticipation of specific phenotypes of calcium channels in different tissues, it is not surprising that the chemically heterogeneous CEB and even different drugs within one class exhibit a wide variety of affinity for the respective target tissues. Three classes of CEB, with each their own receptor binding site[11], can be used as antihypertensive drugs: the dihydropyridines or nifedipine-like drugs, the diphenylalkylamines or verapamil-like drugs, and the benzothiazepines or diltiazem-like drugs. In contrast to the latter two classes, dihydropyridines do not exhibit negative chronotropic or dromotropic effects at the doses that are used in humans[14]. Moreover, nifedipine and particularly the newer dihydropyridines such as felodipine have a high vascular selectivity[15] and hence a more specific vasodilatory action with less negative inotropic effects. Such potent arteriolar vasodilators might be ideal antihypertensive drugs because they can correct the elevated peripheral resistance which characterizes established hypertension[16]. However, when using the directly acting vasodilators such as diazoxide and minoxidil, the decrease in blood

pressure is partly offset by both an increase in cardiac output resulting from activation of the baroreceptor reflex and renal sodium retention[17]. Dihydropyridine CEB also activate the baroreceptor reflex[18], but the degree of activation appears to be less than with other vasodilators[10] and might be largely prevented by an administration schedule which results in a slow, gradual rise of plasma drug levels[19]. Chronic treatment with CEB even resets the baroreceptor reflex within one week, thus allowing sustained blood pressure reduction without persistent tachycardia[18,20].

Natriuretic effects of calcium entry blockers

The compensatory renal sodium retention that is observed during treatment with directly acting vasodilators[21,22] can be explained, according to Guyton, by the pressure-natriuresis relationship which is determined by the kidneys[23]: at a fixed sodium intake, a decrease in blood pressure will lead to sodium retention until the equilibrium pressure, which belongs to that sodium intake, is restored. In this perspective, only a concomitant influence of an antihypertensive drug on the kidneys, which changes the pressure-natriuresis relationship, can prevent sodium retention when blood pressure is reduced. Directly acting vasodilators apparently lack such an effect. It is therefore quite remarkable and of great clinical importance that CEB change the pressure-natriuresis relationship in such a way that blood pressure is reduced without sodium retention[24]. CEB and directly acting vasodilators indeed have opposite effects on renal sodium excretion, as has been demonstrated nicely in salt-loaded normotensive and hypertensive rats where minoxidil induced sodium retention in sharp contrast to the acute natriuresis with equipotent hypotensive doses of the dihydropyridine nitrendipine[25]. In humans, an acute natriuretic effect of nifedipine was already demonstrated in 1972[26], but it took 10 years before CEB received serious attention as effective vasodilators devoid of sodium retaining properties[27]. The acute natriuretic action of CEB was later confirmed in many studies in healthy volunteers and hypertensive patients as reviewed by others[28,29]. Only very high doses of CEB which decrease blood pressure more than 20 % can induce sodium retention[30,31], probably by reducing renal perfusion pressure below the autoregulation limit for glomerular filtration. The natriuretic effect of CEB thus has been well established, but the mechanisms responsible for this effect are still largely unknown[29].

Although CEB induce diuresis and natriuresis, they lack all possible side effects of diuretics. CEB do not cause an increase of urinary potassium excretion[28], and the use of these drugs is therefore not associated with hypokalemia. A possible explanation for this beneficial effect might be that CEB can inhibit aldosterone release by the adrenal glands[32] in contrast to most diuretics. CEB do not induce hyperuricemia[33]. They can even increase urinary urate excretion[27,34] probably as a consequence of decreased proximal tubular sodium reabsorption[35]. The usual clinical dosages of CEB also induce no or only minor, transient changes of lipid and glucose homeostasis[36], despite the fact that insulin release partly depends on calcium influx.

Previous work from our department has demonstrated the effectiveness of felodipine in treating hypertension in patients with moderate to severe renal insufficiency[37]. In such patients, most CEB can be used without major dose adjustments, because degradation of these lipophilic and highly protein bound drugs occurs almost exclusively through metabolisation in the liver[10,38]. Clearance of the active drug therefore does not depend on renal function. In patients with renal insufficiency, sodium and water retention can be a major problem and probably contributes to the occurrence of hypertension as indicated by animal studies[39]. Under these circumstances, a natriuretic effect of a vasodilator might be of special value.

The acute sodium losses with CEB appear to be maintained on the long term, as indicated by sodium balance studies after initiation or discontinuation of these drugs [40,41]. This chronic sodium loss has initiated the discussion whether sodium restriction and/or diuretics will augment the antihypertensive effects of CEB[42,43]. Experimental evidence even supports an enhanced antihypertensive effect of CEB during high salt intake, which might be explained by a greater natriuretic effect[44] or an enhanced reactivity of smooth muscle cells during high salt intake[45].

Edema formation with calcium entry blockers

In view of their natriuretic effect, it is quite surprising that ankle edema is one of the major side effects of CEB with an estimated incidence of up to 10 % for the dihydropyridines[10]. The higher incidence with dihydropyridines than with other classes of CEB suggests a relation with the higher vascular selectivity and hence the more pronounced peripheral vasodilating effects of this class of CEB. Edema formation is also a common side effect of other

potent arteriolar vasodilators such as minoxidil[21] and has there been ascribed to renal sodium retention secondary to blood pressure reduction[17]. However, the natriuretic effects of CEB have cast doubt on this explanation. Therefore, it has been suggested that ankle edema with CEB and possibly also with other arteriolar vasodilators is a direct, local consequence of the peripheral vasodilation and thus precedes sodium retention, if that occurs.

Outline of the thesis

In the first part of this thesis (chapters 2-5), we have evaluated several possible mechanisms that might explain the natriuretic effect of CEB. To that end, several renal clearance studies with continuous felodipine infusion were performed in healthy volunteers. Chapter 2 discusses the possibility that the natriuretic action of felodipine is due to reversal of the sodium retaining effect of angiotensin-II. This hypothesis originated from previous work in our department that had demonstrated that felodipine prevented the sodium retention of exogenous angiotensin-II[46]. As a logical extension to these observations, we have evaluated the possibility of a similar interaction between CEB and endogenous angiotensin-II. Subsequently, we have studied the effects of felodipine on urinary potassium excretion, and more specifically the role of aldosterone in the remarkable absence of kaliuresis during CEB-induced natriuresis. Finally, the role of the intrarenal natriuretic hormone dopamine in the natriuretic action of CEB was studied.

The second part of the thesis (chapters 6 and 7) describes two conditions in hypertensive patients in which the natriuretic effects of CEB might play a special role. First, we studied whether felodipine also induced natriuresis in hypertensive patients with renal disease and different degrees of renal insufficiency. Next, the value of sodium restriction in the antihypertensive treatment with CEB was determined in patients with essential hypertension.

The final part of the thesis (chapters 8 and 9) focuses on the edema formation with CEB and other vasodilators. A new, accurate method for measuring foot volume as an exact measure for the degree of ankle edema is described. Subsequently, we studied the acute effects of nifedipine and diazoxide on foot volume and natriuresis in healthy volunteers.

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CHAPTER 2

Is natriuresis on felodipine due to reversal of the renal effects of angiotensin-II ?

Henk W. van Hamersvelt, Henk E. Sluiter, Jack F.M. Wetzels, Robert A.P. Koene, and Frans Th.M. Huysmans.

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ABSTRACT

Felodipine induces natriuresis, possibly by renal hemodynamic and/or tubular effects. Theoretically, reversal of the sodium-retaining effect of angiotensin-II (Ang II) could be involved. Therefore, we administered felodipine during Ang II infusion and during suppression of endogenous Ang II production in two double-blind studies in healthy volunteers.

First, a gradually increasing dose of Ang II was infused during felodipine or solvent infusion. Before starting Ang II, felodipine had lowered renal vascular resistance (RVR) and filtration fraction (FF), and simultaneously increased Cl_{Na}. The Ang II-induced rise of mean arterial pressure (MAP) and renal vasoconstriction was partly antagonized and the falls in glomerular filtration rate (GFR) and Cl_{Na} completely abolished by felodipine. The combination of felodipine and 3.0 ng/kg/min Ang II even enhanced natriuresis.

Second, felodipine or solvent was infused after one week of pretreatment with placebo or the angiotensin converting enzyme (ACE) inhibitor ramipril, which reduced MAP and induced renal vasodilation. Ramipril pretreatment did not influence significantly the blood pressure reduction, renal vasodilation, and natriuresis caused by felodipine.

In conclusion, it seems unlikely that the natriuretic effect of felodipine is due to interference with renal effects of endogenous Ang II. The fact that felodipine reverses sodium retention on exogenous Ang II may be explained by interference with systemic and renal hemodynamic effects specific of exogenous Ang II.

INTRODUCTION

Dihydropyridine calcium entry blockers such as felodipine induce vasodilation and have a powerful antihypertensive effect[1]. In contrast to the sodium-retaining effect of other vasodilating drugs[2], calcium entry blockers of different chemical classes all have an acute natriuretic effect[3], possibly related to their common ability to block the voltage-dependent calcium channel. As this natriuresis is not accompanied by consistent changes in glomerular filtration rate (GFR)[4], a decrease in tubular sodium reabsorption must be assumed. Micropuncture studies in whole animals have localized the natriuretic effect of calcium entry blockers to the proximal tubule during hydropenia[5,6] and the distal tubule during salt loading[7-9]. Clearance studies in humans suggest both a proximal and distal tubular site of action[10,11]. Theoretically, a decrease in tubular sodium reabsorption on calcium entry blockers could be due to a direct effect on sodium transport in tubular cells, an indirect effect mediated through changes in renal hemodynamics, or an indirect effect mediated through an interaction with one of the sodium-retaining or natriuretic hormones.

In favor of a direct tubular effect, it has been shown that calcium entry blockers decrease proximal tubular fluid reabsorption during microperfusion[12] and also inhibit sodium reabsorption in both isolated perfused proximal tubules of rabbits[13] and in the isolated toad urinary bladder[14,15], a model for distal renal tubular function. In accordance with the supposed in vivo site of action of calcium entry blockers, this direct effect was more pronounced when the drug was given at the peritubular[12] and the serosal[14] side. However, a direct effect on renal tubular cells cannot be understood readily, since voltage-operated calcium channels have not, as yet, been identified in renal tubular cells[16] and also since a decrease in cytosolic calcium is expected to enhance sodium reabsorption[17]. Moreover, the very high doses (10^{-4} to 10^{-5} M) used in these studies make it difficult to translate the results to man, where indirect mechanisms could be equally or even more important.

In most studies in man[18], renal hemodynamic changes favoring natriuresis could explain at least part of the proximal tubular effect. However, in dogs, calcium entry blockers also induced natriuresis when an aortic clamp prevented any rise in renal blood flow (RBF)[6,19], suggesting that changes in renal hemodynamics cannot be the only factor responsible for the natriuretic effect. Therefore, an interaction of calcium entry blockers with one of the sodium-retaining or natriuretic hormones could be involved as well. As selective administration of a calcium entry blocker into one renal artery increases sodium

excretion only in the corresponding kidney[20], it seems unlikely that a change in systemic secretion of hormones such as ANP plays a major role in the natriuretic effect. Thus, interference with the local, intrarenal secretion of a hormone such as angiotensin II (Ang II)[21,22], or inhibition of the renal effect of this hormone could be involved. Blockade of the physiologic effects of Ang II results in renal vasodilation and a decrease in proximal and distal tubular sodium reabsorption, effects comparable to the renal actions of calcium entry blockers. Moreover, many of the physiologic effects of Ang II depend on the availability of intracellular free calcium[23]. In fact, in animals, calcium entry blockers can prevent both the decrease in renal blood flow and the sodium retention induced by exogenous Ang II[14,24-26]. Therefore, an antagonism of the renal effects of Ang II could be responsible for the natriuretic effect of felodipine. To evaluate this hypothesis, we studied the natriuretic effect of felodipine in healthy volunteers during concomitant infusion with Ang II and also after pretreatment with the angiotensin converting enzyme (ACE) inhibitor ramipril.

METHODS

The interactions between felodipine and Ang II (study A) and between felodipine and the ACE inhibitor ramipril (study B) were examined in two groups of 12 healthy male volunteers, aged 21-35 years. All participants were free of medication, had normal kidney function, and had a blood pressure below 140/90 mm Hg. Dietary sodium intake was restricted to approximately 100 mmol per day in the week preceding each infusion experiment.

Study A

In a double-blind, randomized, crossover fashion, felodipine and placebo (solvent) were infused for 300 minutes on two separate days, with an interval washout period of three weeks. During ongoing felodipine or solvent infusion, a gradually increasing dose of the Ang II analogue val^5-angiotensin-II-asp^1-β-amide (Hypertensin^R, Ciba Geigy) was given for 120 minutes. Felodipine was infused at a rate of 9 μg/minute for the first 40 minutes followed by 6.75 μg/minute for the remaining 260 minutes. One hundred and forty minutes after the start of felodipine or solvent, Ang II was given in increasing doses of 0.3, 1.0 and 3.0 ng/kg/min in periods lasting 40 minutes each. Moderate water

diuresis was established by an oral water load of 625 ml before the experiment and was maintained by infusion of 400 ml of 0.25 % NaCl in 3.3 % glucose per hour. Two urine collections of 40 minutes were made before the Ang II infusion, one during each dose of Ang II, and one after stopping the Ang II infusion. Blood pressure was measured by Arteriosonde (model 1225).

Study B

In a double-blind, randomized, crossover fashion, felodipine was infused for 200 minutes after seven days of oral pretreatment with ramipril, 5 mg twice daily. In two control experiments, felodipine was infused after one week of oral placebo and solvent was infused after ramipril. Washout periods lasted at least three weeks. Felodipine was infused at a rate of 0.15 μg/kg/min (mean 11 μg/min) for the first 40 minutes followed by 0.10 μg/kg/min (mean 7.4 μg/min) for the remaining 160 minutes. Maximal water diuresis was induced by an oral water load of 25 ml/kg and was maintained by infusion of 400 ml of 0.25 % NaCl in 3.3 % glucose per hour and by oral replacement of urinary losses in excess of this infusion rate. Two urine collections of 40 minutes were made before and five during infusion of felodipine or solvent. Blood pressure was measured by Dinamap (model 1846P, Critikon).

Both studies

In both studies, subjects were kept fasting during the infusion experiment and remained supine except for spontaneous voiding. With a continuous infusion technique[27], renal plasma flow (RPF) and GFR were estimated by measurement of renal clearances of PAH and inulin (laevofructosan, Inutest[R]), respectively. PAH, inulin and electrolytes were measured by standard (semi) automated techniques, and felodipine plasma levels by gas chromatography[28]. Clearances of various substances (Cl_x) were calculated by the standard formula $Cl_x=(U_x*V)/P$ and fractional excretions (FE_x) by Cl_x/GFR. RBF was approximated by RPF/(1-Hct), renal vascular resistance (RVR) by 1000*MAP/RBF and filtration fraction (FF) by GFR/RPF.

For statistical analysis, Wilcoxon's rank sum test was used for comparison of baseline values, whereas repeated measures analysis of variance (ANOVA) was used to compare responses to Ang II in study A and responses to felodipine and

solvent infusions in study B. A probability value below 0.05 was considered statistically significant. All results are given as means±SEM.

The study protocol was approved by the hospital Ethics Committee and all volunteers gave written informed consent.

RESULTS

Study A

One of the volunteers had problems with spontaneous voiding, resulting in a very irregular urinary flow rate. Renal clearances were considered unreliable in this subject and his results were excluded from the analysis. All results thus concern the remaining 11 subjects (Table 1). The absolute values before start of Ang II infusion were measured after 140 minutes of infusion with felodipine

Table 1. *Effects of graded Ang II infusion (in ng/kg/min) during solvent and felodipine*

		before Ang II	0.3	1.0	3.0	after Ang II
		abs value	Δ abs			
MAP	S	81±3	+2±1	+5±1^{A}	+11±1^{A}	+4±2
mm Hg	F	75±2	+1±2	+2±1^{D}	+8±1^{A}	+2±1
RBF	S	972±43	-95±32^{B}	-150±35^{A}	-305±31^{A}	-48±31
ml/min	F	1067±42	-20±61	-38±67	-175±54^{A}	-10±55
GFR	S	108±5	-7±4	-5±4	-11±3^{A}	+2±3
ml/min	F	100±4	+4±5	+10±6	+5±5^{D}	+7±5
FF	S	18.8±1.0	+0.7±0.6	+2.2±0.9^{B}	+5.7±0.9^{A}	+1.0±0.6
%	F	16.2±0.8^{D}	+0.9±0.5	+2.3±0.7^{A}	+4.1±0.9^{A}	+1.2±0.4^{B}
RVR	S	83±4	+12±3^{A}	+22±3^{A}	+56±6^{A}	+9±3^{B}
units	F	71±4^{C}	+3±4	+5±5^{D}	+22±5^{A,C}	+3±3
Cl_{Na}	S	0.9±0.1	-0.2±0.1^{B}	-0.3±0.1^{B}	-0.5±0.1^{A}	+0.2±0.1^{B}
ml/min	F	2.8±0.3^{C}	+0.1±0.2	+0.4±0.2^{C}	+1.4±0.4^{A,C}	+1.0±0.2^{A,D}
FE_{Na}	S	0.9±0.1	-0.1±0.1^{B}	-0.2±0.1^{B}	-0.4±0.1^{A}	+0.2±0.1^{B}
%	F	2.8±0.3^{C}	-0.1±0.1	+0.1±0.1^{C}	+1.1±0.3^{A,C}	+0.7±0.2^{B}

Abbreviations: abs value, absolute value before start of Ang II infusion; Δ abs, absolute change from value before Ang II infusion. S, solvent infusion; F, felodipine infusion.

^{A} $P < 0.01$ and ^{B} $P < 0.05$ compared to values before Ang II infusion.

^{C} $P < 0.01$ and ^{D} $P < 0.05$ compared to solvent infusion.

or solvent and thus indicate the effects of felodipine without Ang II. Felodipine infusion resulted in a slightly, but not significantly lower MAP ($P=0.08$), a lower RVR and a not significantly higher RBF ($P=0.10$). GFR was not changed, whereas FF was decreased. Felodipine infusion was followed by a threefold rise in Cl_{Na} and FE_{Na}. Felodipine plasma levels increased gradually from 7.1 ± 0.5 nmol/litre at 40 minutes to 11.6 ± 0.4 at 260 minutes after start of felodipine infusion.

Graded Ang II infusion during solvent resulted in a dose-related rise of blood pressure, renal vasoconstriction, and sodium retention (Table 1). GFR was reduced less than RBF, leading to an increase in FF. The degree of sodium retention (Cl_{Na}) was associated with the decrease in RBF during the two higher doses of Ang II ($r=0.72$ and $r=0.70$, respectively, both $P<0.02$).

Felodipine infusion partly antagonized these effects of Ang II (Table 1). During infusion of 1.0 ng/kg/min Ang II, felodipine prevented the rise in MAP and RVR, tended to antagonize the decrease in GFR ($P=0.08$), and abolished the sodium retention caused by Ang II. During infusion of 3.0 ng/kg/min Ang II, felodipine did not prevent the rise in MAP and only partly antagonized the Ang II-induced rise of RVR and decrease in RBF ($P=0.058$), but abolished the Ang II-induced decrease in GFR. Moreover, at this dose of Ang II, felodipine not only prevented the sodium retention by Ang II, but even enhanced sodium excretion by 50 ± 13 %. Felodipine infusion did not influence the Ang II-induced rise of FF.

Study B

After completion of this study, one subject was excluded from the analysis, because his serum PAH and inulin levels could not be determined accurately due to technical problems. All results thus concern the remaining 11 subjects (Table 2). Baseline values were measured before starting felodipine or solvent infusion and thus indicate the effect of pretreatment *per se*.

After seven days of pretreatment with ramipril, blood pressure reduction, renal vasodilation, and a reduction of ACE activity to 11 ± 1 % were observed. Comparison of the effects of solvent and felodipine infusions after ramipril shows that ramipril pretreatment did not prevent the effects of felodipine on renal hemodynamics and sodium excretion. After placebo, but also after ramipril pretreatment, felodipine infusion reduced blood pressure, induced renal vasodilation, decreased FF, and increased FE_{Na}. The felodipine-induced

Table 2. *Effects of felodipine and solvent infusion after pretreatment with ramipril or placebo*

		Rami + Solv	Rami + Felo	Plac + Felo
MAP	baseline	86 ± 2	86 ± 2	90 ± 2[A]
mm Hg	Δ abs (inf)	0 ± 1[B]	-5 ± 1	-7 ± 1
RBF	baseline	1108 ± 51	1079 ± 46	957 ± 56[B]
ml/min	Δ abs (inf)	-58 ± 28[A]	+126 ± 35	+156 ± 21
GFR	baseline	107 ± 4	111 ± 2	106 ± 3
ml/min	Δ abs (inf)	-1 ± 2	+3 ± 2	+2 ± 1
FF	baseline	17.0 ± 0.7	18.0 ± 0.8	19.8 ± 1.3
%	Δ abs (inf)	+0.5 ± 0.5[A]	-1.3 ± 0.4	-2.4 ± 0.4[A]
RVR	baseline	68 ± 4	71 ± 4	86 ± 6[A]
arbitrary units	Δ abs (inf)	+4 ± 2[A]	-10 ± 2	-18 ± 3[B]
FE_{Na}	baseline	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
%	Δ abs (inf)	+0.2 ± 0.1[A]	+1.2 ± 0.2	+1.7 ± 0.2

Abbreviations: rami and plac, ramipril and placebo pretreatment; solv and felo, solvent and felodipine infusions; Δ abs (inf), mean absolute changes from baseline during drug infusions.
[A] $P < 0.01$ and [B] $P < 0.05$, compared to rami + felo.

decreases of FF and RVR were significantly diminished by ramipril pretreatment. The negative influence of ramipril on felodipine-induced natriuresis failed to reach statistical significance ($P=0.07$) and ramipril had no significant effect ($P>0.10$) on the felodipine-induced decrease in MAP and increase in RBF. GFR was not changed by ramipril nor by felodipine. Correlation analysis showed that a lower baseline MAP after ramipril was associated with a more pronounced inhibition of natriuresis on felodipine ($r=0.67$, $P=0.02$). Felodipine plasma levels increased gradually from 8.2 ± 3.1 nmol/litre at 40 minutes to 12.7 ± 1.7 at 200 minutes after placebo pretreatment and from 7.1 ± 2.2 to 12.4 ± 1.6 after ramipril.

DISCUSSION

An interaction with the renal tubular effect of Ang II could explain the natriuretic action of calcium entry blockers. If such an interaction were present, one would expect that calcium entry blockers prevent the sodium retention

induced by physiologic amounts of exogenous Ang II and also that suppression of endogenous Ang II production prior to treatment with a calcium entry blocker would interfere with the acute natriuretic action of this drug. Our two studies show that felodipine prevented the sodium retention induced by exogenous Ang II, whereas suppression of Ang II production by ramipril had no significant effect on the acute natriuresis induced by felodipine. This apparent discrepancy cannot be explained by differences in felodipine dose or sodium intake, since felodipine plasma levels and daily urinary sodium excretion rates (data not shown) were comparable in the two studies. Theoretically, an acute natriuretic effect of ramipril[29] could have masked an inhibitory influence of ramipril on felodipine-induced natriuresis. However, the effects of felodipine were studied after one week of pretreatment with ramipril and thus at a time when sodium balance should have been restored. Indeed, baseline sodium excretion rate was not changed by ramipril and hardly any change in sodium excretion was observed during solvent infusion after ramipril. Therefore, we must assume that the natriuretic effect of felodipine does not depend mainly on interference with the sodium-retaining effect of endogenous Ang II at renal tubular level. Moreover, the minor and statistically insignificant negative influence of ramipril on felodipine-induced natriuresis was related to the ramipril-mediated reduction in baseline blood pressure. This suggests that ramipril might have blunted the natriuretic action of felodipine through reduction of renal arterial pressure (RAP). In anesthetized dogs, reduction of RAP by an aortic clamp also diminished the natriuretic effect of the calcium entry blocker diltiazem[19].

If we assume that natriuresis on felodipine does not depend mainly on the presence of endogenous Ang II, the question remains why felodipine not only prevented sodium retention by exogenous Ang II, but even enhanced natriuresis at the highest dose of Ang II. In this respect, it is important to realize that exogenous Ang II does not mimic the effects of Ang II formed intrarenally, since relatively high doses of Ang II have to be given systemically in order to reach relevant changes in intrarenal levels[21]. Intravenously administered Ang II increases systemic blood pressure and thus RAP. This rise of RAP leads to a reflex myogenic preglomerular arteriolar vasoconstriction[30-33]. Moreover, a direct effect of exogenous Ang II on the preglomerular vessel might contribute to this constriction[32], although Ang II acts preferentially on the postglomerular arteriole[30]. Thus, despite an increase in RAP, Ang II infusion decreases RBF due to pre- and postglomerular vasoconstriction, and increases FF. This renal vasoconstriction is probably in part responsible for the sodium-retaining effect of Ang II, since the decrease in RBF is related to the decrease in sodium

excretion during Ang II. In accordance with other studies[14,24,25,34,35], we observed that felodipine not only attenuated the rise in blood pressure on Ang II, but also blunted the rise of RVR. This last effect might explain part of the inhibition of the sodium-retaining effect of Ang II by felodipine. Animal studies showed that calcium entry blockers prevent pressure-induced vasoconstriction in isolated interlobular arteries[31] and also in preglomerular arterioles of isolated perfused hydronephrotic kidneys[36], whereas they do not affect postglomerular resistance[37]. From this, it can be understood that felodipine not only attenuated the rise in RVR, but also prevented the fall in GFR at all doses of Ang II. Preferential preglomerular vasodilation by felodipine together with the rise in blood pressure at the highest dose of Ang II can explain the enhanced natriuresis during the combination of Ang II and felodipine. Similar observations of enhanced natriuresis during Ang II infusion were made in anesthetized dogs, when blood pressure increased and renal vasoconstriction was prevented by simultaneous infusion of various vasodilators into the renal arteries[38]. Therefore, the reversal of Ang II-induced sodium retention by felodipine can be explained without assuming a specific interaction at renal tubular level. However, in contrast to other types of vasodilators, calcium entry blockers also induce natriuresis when administered systemically, indicating a specific effect of these drugs on sodium excretion.

In summary, it seems likely that calcium entry blockers interfere with some actions of exogenous Ang II, especially the rise in blood pressure and preglomerular vasoconstriction. Although we cannot exclude that felodipine specifically reverses the sodium-retaining effect of exogenous Ang II at renal tubular level, most of our observations on sodium excretion can also be explained by an indirect effect of felodipine on the myogenic preglomerular vasoconstriction secondary to the Ang II-induced rise in blood pressure. The natriuretic effect of felodipine still occurs during ACE-inhibition with ramipril, implying that mechanisms other than interference with the tubular effects of endogenous Ang II must be involved in the natriuretic action of calcium entry blockers. Further studies are needed to elucidate the exact mechanisms involved.

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CHAPTER 3

Angiotensin converting enzyme inhibition does not prevent the natriuretic effect of felodipine

Henk W. van Hamersvelt, Jack F.M. Wetzels, Heinrich J. Kloke,

Robert A.P. Koene, and Frans Th.M. Huysmans.

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ABSTRACT

The mechanism of natriuresis with calcium entry blockers such as felodipine is largely unexplained. As these drugs prevent sodium retention following exogenous angiotensin II, the natriuretic effect of felodipine might be due to a similar interaction with endogenous angiotensin II. In such a case, angiotensin converting enzyme inhibition with ramipril should prevent natriuresis with felodipine.

We tested this hypothesis in a randomized, double-blind, crossover study in 12 male volunteers by comparing intravenous felodipine after one week of oral ramipril with felodipine after placebo and with solvent after ramipril. Ramipril pretreatment reduced ACE activity to 11 ± 1 %, lowered the blood pressure and increased renal blood flow. However, ramipril pretreatment did not prevent the pronounced increase in natriuresis and diuresis with felodipine. Fractional sodium excretion only tended to increase less during felodipine after ramipril than during felodipine after placebo (absolute changes of $+1.2 \pm 0.2$ % and $+1.7 \pm 0.2$ %, respectively, $P=0.07$). Ramipril did not influence felodipine-mediated blood pressure reduction and renal vasodilation.

In conclusion, ACE inhibition has no significant effect on the natriuretic and hemodynamic effects of felodipine, suggesting that mechanisms other than interaction with endogenous angiotensin II are involved in these effects of calcium entry blockers.

INTRODUCTION

Dihydropyridine calcium entry blockers such as felodipine have proven to be effective antihypertensive drugs[1]. In contrast to other vasodilators, calcium entry blockers of different chemical classes all have a natriuretic effect[2], which possibly contributes to their antihypertensive action[3]. Micropuncture and microperfusion studies have documented inhibition of tubular sodium reabsorption in either the proximal[4-6] or the distal[7-9] renal tubule, but the mechanism responsible for these effects remains to be identified. Theoretically, a direct effect on tubular sodium transport, renal hemodynamic changes or an interaction with one of the natriuretic or sodium-retaining hormones could be involved. Sodium retention induced by exogenous angiotensin II[10] can be prevented by calcium entry blockers, both in animals[11-14] and in humans[15]. Since endogenous angiotensin II probably has a sodium-retaining effect similar to exogenous angiotensin II [16,17], it is possible that the natriuretic action of calcium entry blockers is due to an interaction with endogenous angiotensin II. Therefore we studied the natriuretic effect of felodipine after suppression of angiotensin II production by the angiotensin converting enzyme (ACE) inhibitor ramipril.

METHODS

Subjects

Initially, 12 healthy male volunteers were recruited. Because of adverse experiences, two of these subjects were replaced during the study. All participants were free of medication. Ages ranged from 21 to 35 years (mean of 25 years) and all had a blood pressure $<140/90$ mm Hg(mean$\pm$SD of $127\pm11/80\pm8$ mm Hg), and normal length (183 ± 8 cm) and body weight (74 ± 8 kg).

The study protocol was approved by the hospital Ethics Committee and all volunteers gave written informed consent.

Study protocol

Each subject received a 200 min infusion with felodipine after 7 days of oral pretreatment with the ACE inhibitor ramipril, 5 mg, given twice daily to obtain powerful ACE inhibition throughout 24-hours. In two control experiments, each subject received a placebo infusion (solvent) after pretreatment with ramipril and a felodipine infusion after pretreatment with oral placebo. A double-blind, randomized, two-way crossover design was used with felodipine infusion after ramipril always second in order. Treatment periods were separated by wash-out intervals of 3 weeks. During treatment a diet containing approximately 100 mmol sodium per day was prescribed and compliance was tested by measuring creatinine and sodium in 24-hour urine samples. The evening preceding each clearance study, 600 mg of lithium carbonate (16.2 mmol of lithium) was given orally. Subjects were asked to refrain from smoking and the use of alcohol for the last 24 hours preceding clearance studies and also from caffeine-containing beverages for the last 8 hours.

On the eighth day of oral pretreatment, 1 hour after a light breakfast, infusion experiments took place between 8.30 h and 16.30 h. Upon arrival, body weight was measured and the last ramipril or placebo capsule was given. Maximal water diuresis was induced by an oral water load of 25 ml/kg of body weight, resulting in a urinary osmolality of 65 mOsm/kg (range of 51 to 89 mOsm/kg) and a urinary flow rate of 14 ml/min (range of 9 to 19 ml/min). Thereafter, 0.25 % sodium chloride in 3.3 % glucose was infused at a rate of 400 ml/hr to maintain diuresis and to compensate for sodium losses observed during placebo[18]. Urinary volume losses in excess of 400 ml/hr were replaced orally by tap water. Subjects remained supine except for spontaneous voiding. With a continuous infusion technique[15], the renal plasma flow (RPF) and glomerular filtration rate (GFR) were estimated by measurement of renal clearances of *p*-aminohippuric acid (PAH) and inulin (laevofructosan, Laevosan-Gesellschaft, Linz, Austria), respectively. After 90 min of equilibration, two baseline urine collections of 30 min were made before starting felodipine or solvent infusion. To obtain stable plasma levels in the therapeutic range, 0.15 μg/kg/min of felodipine was infused for the first 40 min followed by 0.10 μg/kg/min for the remaining 160 min[15] (cumulative dose 1.60 ± 0.15 mg, mean$\pm$SD). Five further urine collections of 40 min were made with blood samples drawn at the beginning and the end of each urine collection period. In blood and urine samples, PAH, inulin,

sodium, potassium, chloride, urate, and phosphate concentrations were measured by standard (semi)automated techniques, whereas lithium was determined by atomic absorption spectrophotometry and osmolality by freezing point depression. In blood samples hematocrit (Ht) was determined by routine Coulter counter, ACE activity by a colorimetric method (ACE-color, Fujirebio, Tokyo, Japan)[19] and plasma renin activity (PRA) and plasma aldosterone concentration (PAC) by radioimmunoassay[20,21]. Felodipine was determined by gas chromatography[22] and ramiprilat, the active metabolite of ramipril, by radioimmunoassay[23]. Blood pressures and pulse rates were recorded at 2 min intervals by an automatic device (Dinamap model 1846P, Critikon, Tampa, FL, USA), which directly measures the mean arterial pressure (MAP).

For blood pressure data, the mean values of ten consecutive readings in the middle of each urine collection period were calculated. Of the various substances (s), clearances (Cl_s) and fractional excretions (FE_s) were calculated according to the standard formulas[24]. The renal blood flow (RBF) was approximated by RPF/(1-Ht) (ml/min), the renal vascular resistance (RVR) by 1,000*MAP/RBF (arbitrary units) and the filtration fraction (FF) by (GFR/RPF)*100 (%). The GFR, RPF, and RBF were adjusted to a standard body surface area of 1.73 m^2. In the absence of renal vein samples, we could not correct the RPF and RBF for changes in the extraction ratio's of PAH. The free water clearance (Cl_{H2O}) was calculated as urinary flow rate (V) minus the osmolal clearance (Cl_{osm}). The fractional proximal sodium reabsorption (FPR_{Na}) and fractional distal sodium reabsorption (FDR_{Na}) were calculated as follows:

a) by the lithium clearance method[25]:

$$FPR_{Na}(Li) = (1\text{-}Cl_{Li}/GFR)*100\ (\%).$$

$$FDR_{Na}(Li) = (1\text{-}Cl_{Na}/Cl_{Li})*100\ (\%).$$

b) by the free water clearance method[24]:

$$FPR_{Na}(H_2O) = (1\text{-}(U_{Na+K}*V/P_{Na}+Cl_{H2O})/GFR)*100\ (\%).$$

$$FDR_{Na}(H_2O) = Cl_{H2O}/(Cl_{Cl}+Cl_{H2O})*100\ (\%).$$

Statistical analysis

For evaluation of the influence of pretreatment *per se* on baseline values measured just before starting felodipine or solvent infusion, only ramipril preceding felodipine infusion was compared to oral placebo.

For evaluation of the effect of ramipril on felodipine-induced changes, felodipine infusion after ramipril was compared, on the one hand, to solvent infusion after ramipril and, on the other hand, to felodipine infusion after placebo. Absolute changes from baseline were compared, except for hormonal parameters, where percentage changes were used. For the pharmacokinetic parameters, areas under the curve were compared.

Statistics were performed with SAS (statistical analysis system) software using two tailed Wilcoxon's test for paired data and repeated measures analysis of variance (ANOVA). Correlation coefficients were calculated according to Spearman. A probability value less than 0.05 was considered statistically significant. Results are given as means $\pm$ SEM.

RESULTS

As indicated earlier, two subjects withdrew because of adverse experiences: one because of fainting on the second day of ramipril treatment and the other because of vomiting during felodipine infusion. Because of technical problems, serum inulin and PAH levels of one subject could not be determined. Therefore, data analysis was restricted to the remaining 11 subjects.

On the last 2 days of oral pretreatment, urinary sodium excretions during

Table 1. *Systemic hemodynamic parameters after oral pretreatment and during drug infusions.*

	Blood pressure (*mm Hg*)			HR (*beats/min*)
	Systolic	Diastolic	MAP	
Baseline values after pretreatment				
Placebo	121 $\pm$ 2	72 $\pm$ 3	90 $\pm$ 2	53 $\pm$ 2
Ramipril	115 $\pm$ 2^{A}	67 $\pm$ 3^{A}	86 $\pm$ 2^{A}	53 $\pm$ 2
Mean values during drug infusions				
Ramipril + solvent	116 $\pm$ 2	66 $\pm$ 3^{C}	86 $\pm$ 2^{C}	53 $\pm$ 2^{C}
Ramipril + felodipine	114 $\pm$ 2	62 $\pm$ 2	81 $\pm$ 2	60 $\pm$ 2
Placebo + felodipine	117 $\pm$ 2	64 $\pm$ 2^{B}	83 $\pm$ 2^{B}	62 $\pm$ 3

MAP = mean arterial pressure; HR = heart rate.

$^{A}P < 0.01$ compared to oral placebo; $^{B}P < 0.05$ and $^{C}P < 0.01$ compared to ramipril + felodipine

ramipril (81±7 mmol/day) and placebo (77±12) did not differ, indicating a good adherence to the diet. Also, potassium excretion was the same during ramipril (94±7 mmol/day) and placebo (94±5). Body weight was unchanged by pretreatment with ramipril (72.8± 2.1 kg) compared to placebo (72.9± 2.2).

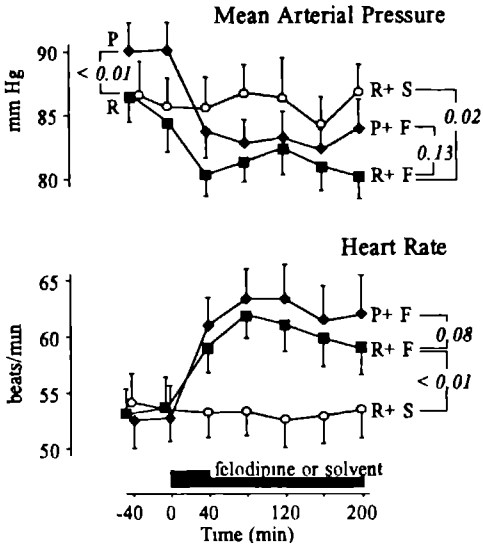

Figure 1. Systemic hemodynamics before (baseline) and during drug infusions. P=placebo; R=ramipril; R+S=ramipril followed by solvent (o); P+F=placebo followed by felodipine (♦); R+F=ramipril followed by felodipine (■); P values on the left: comparison of baseline values; P values on the right: comparison of absolute changes from baseline induced by drug infusion.

Systemic hemodynamics

The baseline blood pressure was lower after ramipril than after oral placebo, whereas the heart rate was the same (Table 1). Felodipine infusion caused a significant decrease in the MAP when compared to solvent infusion (Fig. 1). The decreases in MAP with felodipine were similar after pretreatment with either ramipril or placebo (-5±1 and -7±1 mm Hg, respectively, P= 0.13). Ramipril had only a minor, statistically insignificant effect on the felodipine-induced increase in heart rate (Fig. 1, +7±1 compared to +10±1 beats/min on felodipine after placebo, P=0.08).

Renal hemodynamics

Ramipril pretreatment increased the RBF, but did not change the GFR and FF (Table 2). In comparison to solvent infusion, felodipine infusion increased the RBF and decreased the RVR (both $P<0.01$; Fig. 2 and Table 2). The increases in RBF during felodipine after either ramipril or placebo were comparable (+126±35 compared to +156±21 ml/min/1.73 m^2; P=0.20). However, the decrease in RVR was less pronounced during felodipine after

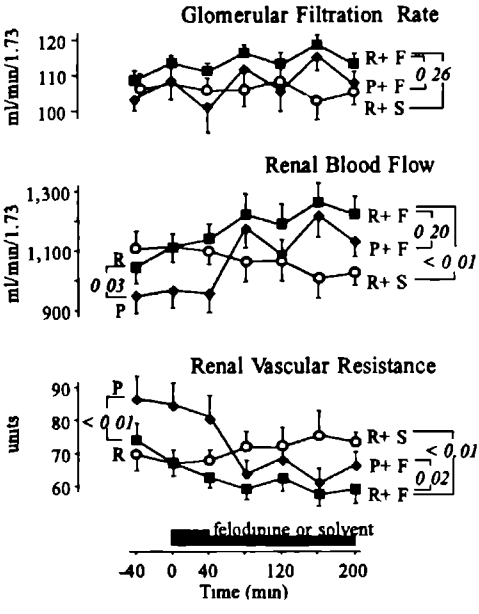

Figure 2. Renal hemodynamics before and during drug infusions. See Figure 1 for abbreviations and explanation of symbols.

ramipril than during felodipine after placebo (-10±2 and -18±3 arbitrary units, respectively; $P=0.02$). The GFR did not change during felodipine and solvent infusions (Fig. 2). In comparison to solvent infusion, felodipine infusion after ramipril lowered the FF (+0.5±0.5 and -1.3±0.4 %, respectively; $P<0.01$). An even larger decrease ($P<0.01$) in FF was found on felodipine after placebo (-2.4± 0.4 %).

Diuresis and electrolyte excretions

The baseline urinary flow rate and fractional excretions of sodium, chloride and potassium were similar after ramipril and placebo pretreatment (Table 3). After ramipril pretreatment, felodipine infusion still induced a pronounced increase of natriuresis and diuresis when compared to solvent infusion (Fig. 3). Moreover, the natriuretic and diuretic effects of felodipine after either ramipril or placebo did not differ significantly ($P=0.07$ and $P=0.46$, respectively; Fig. 3 and Table 3). A positive correlation ($r=0.67$, $P=0.02$) was found between the ramipril-mediated reduction in baseline MAP and the ramipril-induced change in natriuresis with felodipine. Changes in FE_{Cl} paralleled changes in FE_{Na} and only small and insignificant changes in FE_K were observed (Table 3).

Table 4 gives data on segmental tubular sodium reabsorption. FE_{PO4} increased to the same extent during solvent and the two felodipine infusions. Compared to the solvent infusion, felodipine infusion induced an increase in FE_{URA} and decreases in FPR_{Na} as well as FDR_{Na}. Calculated by the Cl_{Li} method, FPR_{Na} decreased less during felodipine after ramipril than during felodipine after placebo, but this difference was not found with FPR_{Na} calculated by Cl_{H2O} method ($P=0.09$). Similar decreases in FDR_{Na} were observed during felodipine infusions after ramipril and placebo.

Table 2. *Renal hemodynamic parameters after oral pretreatment and during drug infusions*

	RBF *ml/min* (*1.73 m²*)	GFR *ml/min* (*1.73 m²*)	FF %	RVR *units*
Baseline values after pretreatment				
Placebo	957±56	106±3	19.8±1.3	86±6
Ramipril	1,079±46[A]	111±2	18.0±0.8	71±4[B]
Mean values during drug infusions				
Ramipril + solvent	1,050±52[D]	106±4	17.5±0.9	72±4[D]
Ramipril + felodipine	1,205±58	114±2	16.8±0.8	60±3
Placebo + felodipine	1,113±50	108±4	17.4±1.1	68±4[C]

See text for abbreviations

[A]$P<0.05$ and [B]$P<0.01$ compared to oral placebo.

[C]$P<0.05$ and [D]$P<0.01$ compared to ramipril + felodipine

Table 3. *Urinary flow rate and fractional electrolyte excretions, baseline values and absolute changes from baseline during drug infusions.*

Pretreatment:	Rami	Rami	Plac	*P* values	
Infusion:	Solv	Felo	Felo	A	B
FE_{Na} *(%)*					
Baseline	0.8±0.1	0.6±0.1	0.7±0.1		0.97
Δ abs (inf)	+0.2±0.0	+1.2±0.2	+1.7±0.2	<0.01	0.07
FE_K *(%)*					
Baseline	9.8±1.4	9.8±1.6	11.2±1.6		0.37
Δ abs (inf)	-2.1±0.8	-0.3±1.0	+0.2±0.9	0.15	0.15
FE_{Cl} *(%)*					
Baseline	0.7±0.1	0.6±0.1	0.5±0.1		0.52
Δ abs (inf)	-0.1±0.0	+1.1±0.3	+1.7±0.3	<0.01	0.12
Urinary flow rate *(ml/min)*					
Baseline	14.6±0.8	14.5±0.8	13.8±0.8		0.20
Δ abs (inf)	-0.6±0.5	+4.8±0.7	+5.6±0.8	<0.01	0.46

Rami=ramipril; Felo=felodipine; Plac=oral placebo; Solv=solvent infusion; Δ abs (inf)=absolute changes from baseline during drug infusions.

See text for other abbreviations.

P values A: comparison of ramipril+solvent to ramipril+felodipine.

P values B: comparison of placebo+felodipine to ramipril+felodipine.

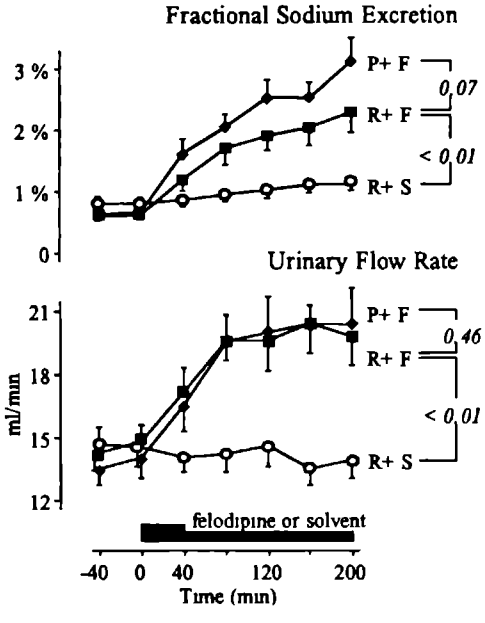

Figure 3. Natriuresis and diuresis before and during drug infusions. See Figure 1 for abbreviations and explanation of symbols.

Hormonal parameters

Ramipril reduced ACE-activity to 11±1 % before and to 13±1 % of pretreatment level during drug infusions. The baseline PRA was higher after ramipril pretreatment (5.6±0.6 compared to 1.1±0.1 nmol/L/h after placebo; $P<0.01$). The baseline PAC was not changed by pretreatment (0.43±0.05 nmol/L after ramipril and 0.55±0.07 nmol/L after placebo; $P=0.41$). As observed in the course of similar clearance studies without any drugs (personal observations), the PRA and PAC decreased during solvent infusion by 36±4 and 30±7 %, respectively. During felodipine after ramipril PRA decreased only by 15± 8 %, i.e., significantly less ($P<0.01$) than during solvent infusion. During felodipine after placebo, the PRA even increased by 16±10 % ($P<0.01$ compared to felodipine after ramipril). The decrease of PAC observed during solvent was also found during felodipine after ramipril (-30±5 %) and during felodipine after placebo (-38±4 %), thereby leading to comparable values for PAC during felodipine infusions (0.30±0.03 and 0.33±0.04 nmol/L, respectively; $P=0.59$).

Pharmacokinetics

There was no difference in ramiprilat plasma levels during felodipine and solvent infusions (Fig. 4). Also, felodipine plasma levels after ramipril and after placebo pretreatment were comparable ($P=0.11$). Both felodipine and ramiprilat plasma levels were in the therapeutic range[26,27].

Table 4. *Segmental tubular sodium reabsorption, base-line values and absolute changes from baseline during drug infusions.*

Pretreatment:	Rami	Rami	Plac	*P* values	
Infusion:	Solv	Felo	Felo	A	B
FE_{URA} *(%)*					
Baseline	7.8±0.7	6.4±0.4	6.9±0.6		0.52
Δ abs (inf)	-0.2±0.3	+2.0±0.3	+2.4±0.4	<0.001	0.44
FE_{PO4} *(%)*					
Baseline	9.8±1.1	8.7±1.2	8.5±0.9		0.76
Δ abs (inf)	+5.5±1.1	+5.8±1.0	+7.2±1.2	0.34	0.34
FPR_{Na} (Cl_{Li}) *(%)*					
Baseline	72.8±1.2	74.9±0.6	72.9±1.8		0.41
Δ abs (inf)	-0.9±0.6	-5.4±0.4	-8.1±0.9	<0.001	0.02
FDR_{Na} (Cl_{Li}) *(%)*					
Baseline	97.1±0.3	97.6±0.2	97.7±0.3		0.64
Δ abs (inf)	-0.6±0.1	-3.6±0.7	-4.4±0.6	0.001	0.27
FPR_{Na} (Cl_{H2O}) *(%)*					
Baseline	89.6±0.4	90.3±0.5	90.1±0.7		0.83
Δ abs (inf)	+0.2±0.4	-3.3±0.3	-4.6±0.5	<0.001	0.09
FDR_{Na} (Cl_{H2O}) *(%)*					
Baseline	93.4±0.9	93.6±0.9	94.7±0.9		0.37
Δ abs (inf)	+0.2±0.3	-6.8±1.4	-9.8±1.5	<0.001	0.11

See Table 3 and the text for abbreviations.

DISCUSSION

This study deals with the influence of ACE inhibition on the natriuretic effect of a calcium entry blocker. Similar to the interaction between calcium entry blockers and exogenous angiotensin II[11-15], an interaction with the sodium-retaining effect of endogenous angiotensin II could be present and could explain the natriuretic action of these drugs. If this hypothesis were true, suppression of angiotensin II production should inhibit this natriuresis. However, chronic ACE inhibition with ramipril did not abolish the natriuresis caused by felodipine and also did not influence felodipine-induced diuresis and renal vasodilation. Although angiotensin II plasma levels were not measured, the ramiprilat plasma levels and the 90 % ACE inhibition indicate

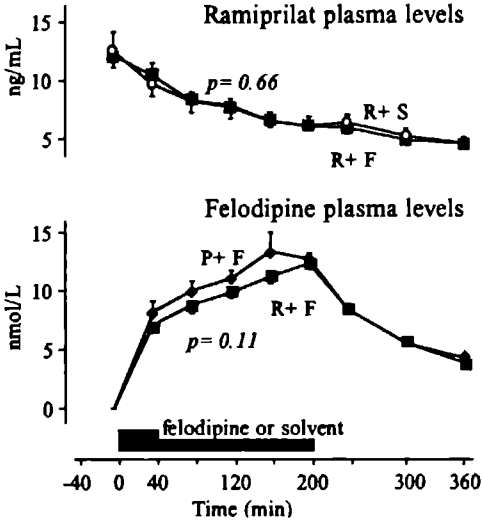

Figure 4. Plasma drug levels during and after drug infusions, the first ramiprilat level measured 150 min after the last gift of ramipril. P values: comparison of area under curve 0-200 min. See Figure 1 for abbreviations and explanation of symbols.

powerful suppression of endogenous angiotensin II production[26,28] during felodipine and solvent infusions. Therefore, we must assume, in contrast to our starting hypothesis, that the natriuretic and renal hemodynamic effects of dihydropyridine calcium entry blockers such as felodipine depend mainly on other factors than interaction with endogenous angiotensin II.

Our data do not exclude the possibility that ACE inhibition slightly inhibits felodipine-induced natriuresis, as the observed P value of 0.07 could indicate a statistical type II error. However, ramipril pretreatment *per se* had lowered the blood pressure and had induced renal vasodilation before the start of felodipine. Moreover, the ramipril-induced reduction in baseline blood pressure was positively correlated to the ramipril-mediated change of felodipine-induced natriuresis. Therefore, if ramipril has any effect on the natriuretic action of felodipine, such an effect could be ascribed to the blood pressure-lowering effect of ramipril and does not necessarily indicate a specific interaction between endogenous angiotensin II and felodipine at the renal tubular level.

The question remains why ACE inhibition has no significant influence on felodipine-induced natriuresis and renal vasodilation, whereas the interaction between exogenous angiotensin II and felodipine had been so prominent in our previous study[15]. This discrepancy cannot be explained by differences in felodipine dose or sodium intake, since felodipine plasma levels and daily urinary sodium excretion rates are comparable in the two studies. It is unlikely that an acute natriuretic effect of ramipril[16] masked an inhibitory influence of ramipril on felodipine-induced natriuresis, since sodium excretion hardly changed during solvent infusion after ramipril. As ACE inhibition not only suppresses angiotensin II production but also retards degradation of

bradykinin, increased bradykinin levels could have influenced our results. Bradykinin has a natriuretic effect[29] and inhibition of bradykinin production by aprotinin prevents natriuresis on calcium entry blockers in animals[30] but not in humans[31]. Thus, theoretically, increased bradykinin levels after ramipril could have facilitated natriuresis on felodipine and thereby could have overridden an inhibitory effect of lowering the levels of endogenous angiotensin II on the natriuretic action of felodipine. In this respect, the use of a specific angiotensin II inhibitor could have provided more definitive answers. Alternatively, the discrepancy between our present results and our previous study[15] could be explained by the differences in effects of intravenous exogenous angiotensin II as opposed to endogenous angiotensin II. Besides the physiologic effects on proximal tubular sodium reabsorption and postglomerular vasoconstriction, intravenously administered angiotensin II might induce an increase in systemic blood pressure, leading to autoregulatory preglomerular vasoconstriction. In the isolated perfused kidney model, calcium entry blockers effectively antagonize such a myogenic preglomerular vasoconstriction[32], whereas they have much less of an effect on postglomerular vasoconstriction induced by angiotensin II[33]. Therefore, it is possible that no specific direct interaction exists between calcium entry blockers and angiotensin II in the kidney. The results obtained in previous studies could be due to an influence of calcium entry blockers on the indirect renal effects of exogenous angiotensin II, i.e., an interaction with the myogenic preglomerular vasoconstriction induced by a rise in blood pressure.

Felodipine infusion increased lithium and urate excretions and decreased the calculated FPR_{Na}, thereby suggesting an inhibition of proximal tubular sodium reabsorption by felodipine. Since lithium and free water clearance methods led to conflicting results regarding an effect of ramipril on the felodipine-induced decrease in FPR_{Na}, our data do not allow firm conclusions on this subject.

Ramipril pretreatment did not influence the blood pressure-lowering effect of felodipine, but tended to blunt the felodipine-induced tachycardia, as has been observed with other combinations of calcium entry blockers and ACE inhibitors[34-36]. Possibly, this inhibitory effect failed to reach statistical significance because of the only modest increase in heart rate, related to the relatively slow rise in plasma drug levels[37].

In conclusion, ACE inhibition with ramipril does not prevent the natriuresis and diuresis on felodipine, suggesting that mechanisms other than interference with the effects of endogenous angiotensin II are involved in the

natriuretic action of dihydropyridine calcium entry blockers. Further studies are needed to elucidate the exact mechanisms involved in the natriuretic effect of calcium entry blockers.

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CHAPTER 4

Exogenous aldosterone antagonizes the distal tubular effects of the calcium entry blocker felodipine

Henk W. van Hamersvelt, Jack F.M. Wetzels, Heinrich J. Kloke, Robert A.P. Koene, and Frans Th.M. Huysmans.

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ABSTRACT

Calcium entry blockers such as felodipine increase natriuresis without increasing kaliuresis. Since these drugs acutely increase plasma renin activity without a concomitant change of aldosterone, inhibition of aldosterone release might explain the absence of kaliuresis.

In a randomized, crossover study in 12 male volunteers we compared the effects of simultaneously administered exogenous aldosterone and felodipine with the effects of either felodipine or aldosterone alone. Felodipine infusion decreased blood pressure, increased renal plasma flow and induced natriuresis without kaliuresis. Aldosterone alone reduced sodium excretion and increased potassium excretion without influencing hemodynamics. Addition of aldosterone to felodipine attenuated its natriuretic effect, and induced a kaliuresis, which clearly exceeded the rise of potassium excretion during aldosterone alone ($\Delta\%$ FE_K $+42\pm12$ and $+7\pm8\%$, respectively, means$\pm$SEM, $P<0.02$).

Our data suggest that felodipine-mediated inhibition of aldosterone release is essential for the absence of kaliuresis with felodipine. In addition, the pronounced kaliuresis with aldosterone during felodipine is in keeping with increased distal sodium delivery due to a proximal tubular action of felodipine.

INTRODUCTION

Calcium entry blockers, especially dihydropyridines such as felodipine, are powerful drugs for the treatment of most forms of hypertension. In contrast to other vasodilating drugs[1], calcium entry blockers induce a diuresis and natriuresis[2] which may contribute to their antihypertensive action. The mechanisms which are responsible for this natriuretic effect remain to be identified. Also the renal tubular site of action is still debated, since micropuncture and microperfusion studies in animals have documented inhibition of sodium reabsorption in either the proximal[3-5] or the distal[6-8] renal tubule. Clearance studies in humans have suggested a proximal as well as a distal tubular site of action[9-13]. In most studies in humans[2,12-15], the natriuretic effect of calcium entry blockers is not accompanied by a rise in potassium excretion, which favours an effect on the distal tubules and especially the cortical collecting ducts where potassium secretion is regulated[16]. In this respect, it is of interest that calcium entry blockade induces a rise of plasma renin activity (PRA) which is not followed by an increase of plasma aldosterone concentration (PAC)[2,17]. Therefore, the absence of kaliuresis during calcium entry blockers could be related to blockade of aldosterone secretion. In addition, inhibition of aldosterone action, or a direct effect on distal tubular sites might be involved. We evaluated these possibilities in healthy volunteers by studying the effects of exogenous aldosterone during infusion of the calcium entry blocker felodipine.

METHODS

Subjects

Initially, twelve healthy male volunteers were recruited. During the study, two of them were replaced because of adverse experiences. All participants were free of medication. Ages ranged from 23 to 36 years (mean 28) and all had a blood pressure below 140/90 (ranges, systolic 108 to 134 and diastolic 68 to 84 mm Hg), normal length (range, 174 to 190 cm), and body weight (range, 62 to 86 kg).

The study protocol was approved by the hospital Ethics Committee and all volunteers gave written informed consent.

Study protocol

Each subject was studied three times in order to compare the simultaneous administration of aldosterone and felodipine with both the administration of aldosterone alone and of felodipine alone. In three clearance experiments separated by washout periods of one week, subjects received combined intravenous infusions of felodipine and aldosterone, of felodipine and placebo (dextrose 5%), and of placebo (solvent) and aldosterone. A randomised, single blind, crossover design was used with the combination of felodipine and aldosterone always second in order and randomisation balanced in blocks of six. Subjects were advised to keep their sodium intake constant and to refrain from licorice on the last three days preceding clearance experiments. Dietary intake was checked by measuring creatinine, sodium and potassium in 24-hour urine samples collected on the last two days preceding clearance studies. Sodium and potassium excretions were corrected for individual mean 24-hour creatinine excretions. On the eve of each clearance study, 600 mg lithium carbonate (16.2 mmol lithium) was given orally. Subjects were asked to refrain from smoking and the use of alcohol for the last 24 hours and from caffeine-containing beverages for the last eight hours preceding the clearance studies.

On study days, the subjects consumed a light breakfast and drank 375 ml tap water. One hour thereafter, clearance experiments took place between 8.30 hr and 15.30 hr. Upon arrival, body weight was measured and water diuresis was induced by an additional oral water load of 15 ml/kg body weight, resulting in a mean urinary osmolality of 66 mOsm/kg (range, 42 to 127). During the whole experiment, 0.25 % sodium chloride in 3.3 % dextrose was infused at a rate of 400 ml/hr to maintain diuresis and to compensate for sodium losses observed previously during similar experiments with placebo[18]. Urinary volume losses in excess of 400 ml/hr were replaced orally by tap water. Subjects remained supine except for spontaneous voiding. With a continuous infusion technique described elsewhere[15] renal plasma flow (RPF) and glomerular filtration rate (GFR) were estimated by measurement of renal clearances of para-aminohippuric acid (PAH) and inulin (polyfructosan, Inutest^{R}, Laevosan-Gesellschaft, Linz, Austria), respectively. After 60 min equilibration, two baseline urine collections of 40 min were made immediately before starting felodipine or solvent infusion. To obtain stable plasma levels in the therapeutic range[10], 0.175 μg/kg/min of felodipine (Plendil^{R}) was infused for the first 40 min, followed by 0.075 μg/kg/min for the remaining 200 min (cumulative dose 1.63 ± 0.15 mg, mean$\pm$SD). Forty minutes after starting felodipine or solvent infusion and after

collection of the third 40 min urine sample, infusion of aldosterone (Aldocorten^{R}, Ciba Geigy, Basel, Switzerland) in 5% dextrose (0.01 mg aldosterone/ml) or 5% dextrose alone was started through a separate intravenous cannula in the upper arm. After an intravenous bolus of 100 μg of aldosterone in 10 min, 0.83 μg/min was infused for the remaining 190 min (cumulative dose 258 μg in 200 min). Five further urine collections of 40 min were made during aldosterone or dextrose infusions. Blood samples were drawn at the beginning and the end of each urine collection period. In blood and urine samples, PAH, inulin, sodium, potassium and chloride concentrations were measured by standard (semi)automated techniques, whereas lithium was determined by atomic absorption spectrophotometry. In blood samples PRA and PAC were determined by radioimmunoassay[19,20] and plasma felodipine levels by gas chromatography[21]. Blood pressures and pulse rates were recorded at four min intervals with an automatic device (Dinamap model 1846P, Critikon, Florida, USA), which directly measures mean arterial pressure (MAP). The mean values of five consecutive readings in the middle of each urine collection period were used for analysis.

Of the various substances (x), clearances (Cl_x), urinary excretions (U_xV) and fractional excretions (FE_x) were calculated according to standard formulas[22] (U_x representing the urinary concentration and V the urinary flow rate). Filtration fraction (FF) was calculated as (GFR/RPF)x100 (%). GFR and RPF were adjusted to a standard body surface area of 1.73 m^2. $U_K/U_{(Na+K)}$ was used as a measure of sodium-potassium exchange in the distal tubule[23]. Fractional proximal sodium reabsorption (FPR_{Na}) and fractional distal sodium reabsorption (FDR_{Na}) were calculated by the lithium clearance method[24]:

$$FPR_{Na} = (1\text{-}Cl_{Li}/GFR)x100\ (\%).$$
$$FDR_{Na} = (1\text{-}Cl_{Na}/Cl_{Li})x100\ (\%).$$

Statistical analysis

Since it takes a latency period of 60-90 min before the effects of aldosterone administration can be observed[25], only the last three urine collection periods were used for evaluation of the effects of aldosterone. For baseline levels, mean values of the first two urine collection periods were calculated. Unless stated otherwise, absolute values and absolute changes from baseline levels were compared. Simultaneous infusion with felodipine and aldosterone was compared to felodipine alone on one hand and to aldosterone alone on the other hand.

Statistics were performed with SAS (Statistical Analysis System) software, using two tailed Wilcoxon's test for simple pairwise comparisons and repeated measures analysis of variance (ANOVA) for all other comparisons. A probability value less than 0.05 was considered statistically significant. Results are presented as means±SEM.

RESULTS

Two volunteers withdrew, one because of personal reasons and the other because of vomiting at the end of felodipine infusion. In total, 12 volunteers completed the whole study protocol.

Baseline data

Body weights and 24-hour urinary water and potassium excretions were comparable preceding the three clearance experiments (Table 1). The 24-hour urinary sodium excretion was lower before infusion of felodipine alone than before simultaneous infusion of felodipine and aldosterone (Table 1), but baseline $U_{Na}V$ and FE_{Na} measured just before felodipine infusions did not differ significantly ($U_{Na}V$: 138±19 and 158±24 μmol/min, P=0.62, Table 2 and FE_{Na}: 0.8±0.1 and 0.9±0.1%, P=0.56, Fig. 2). Baseline excretions of other electrolytes, and baseline levels of systemic and renal hemodynamics were comparable in all three experiments.

Table 1. *Steady state data before clearance studies.*

	Aldo	Felo+Aldo	Felo
Body weight *(kg)*	73.5±1.9	73.4±2.0	73.3±1.9
Urinary volume *(ml/24 hr)*	1612±165	1707±122	1447±123
Urinary sodium *(mmol/24 hr)*	163±14	182±14	139±10*
Urinary potassium *(mmol/24 hr)*	84±6	80±6	76±4

means±SEM. Felo=felodipine; Aldo=aldosterone.
* $P<0.02$ compared to Felo+Aldo.

Systemic and renal hemodynamics

MAP, RPF and GFR are presented in Figure 1. Infusion of felodipine alone lowered MAP by 7±1 %, increased heart rate by 13±3 % and induced renal vasodilation with a 24±4 % rise in RPF (all $P<0.01$ compared to baseline). Felodipine alone did not change GFR (+3±2 %, $P=0.15$), whereas it decreased FF by 16±3 % ($P<0.01$). Aldosterone alone had no effect on systemic and renal hemodynamics. Addition of aldosterone to felodipine did not change the systemic and renal hemodynamic effects of felodipine (all $P\geq0.10$ compared to felodipine alone). Although GFR increased only during simultaneous infusion of felodipine and aldosterone (+7±2 %, $P<0.01$ compared to baseline), this felodipine-mediated change in GFR did not differ significantly ($P=0.16$) from the change during felodipine alone (+3±2 %).

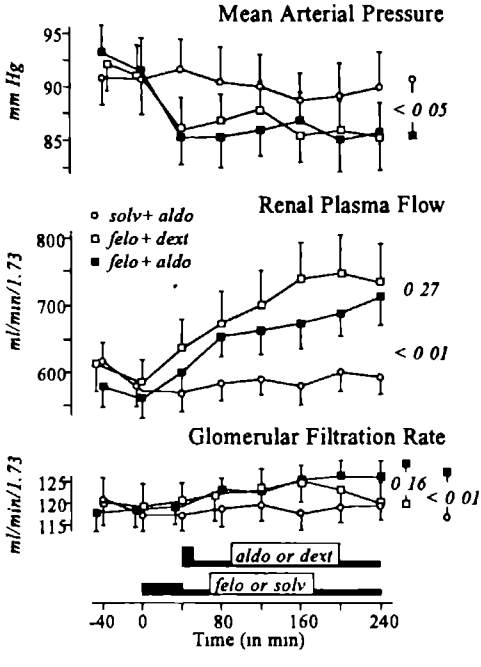

Figure 1. Systemic and renal hemodynamics before (baseline) and during drug and placebo infusions. Felo=felodipine; Solv=solvent; Aldo=aldosterone; Dext=dextrose. P values on the right: comparison of mean absolute changes from baseline during the last three clearance periods.

Diuresis and urinary electrolyte excretions

Felodipine alone had a distinct diuretic and natriuretic effect with a 38±3 % increase in urinary flow rate, a 210±42 % increase in FE_{Na}, and a 208±42 % increase in FE_{Cl} (all $P<0.001$ compared to baseline, Fig. 2). $U_{Na}V$ increased from 138±19 to 435±70 μmol/min and $U_{Cl}V$ from 102±13 to 312±53 μmol/min (both $P<0.001$ compared to baseline, Table 2). In line with our previous observations, the natriuretic effect was not accompanied by changes of U_KV and FE_K ($P>0.10$, Table 2 and Fig. 2). $U_K/U_{(Na+K)}$, FPR_{Na} and FDR_{Na} decreased by 57±4, 11±2, and 5±1 % during felodipine (all $P<0.001$ compa

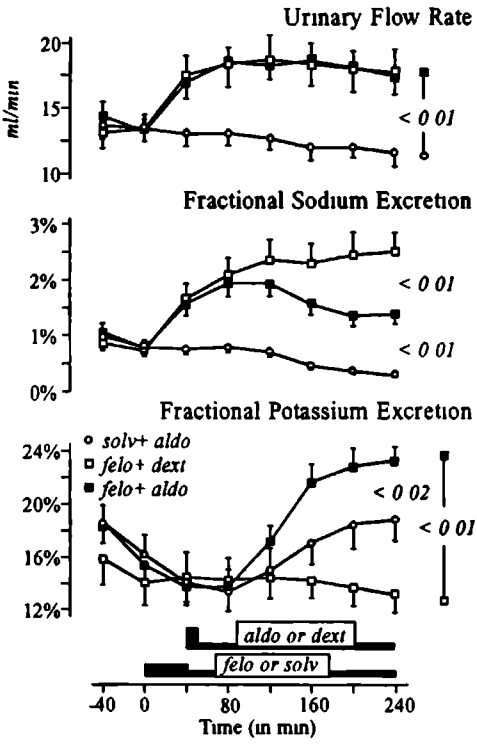

Figure. 2. Diuresis, natriuresis and kaliuresis before (baseline) and during drug and placebo infusions. Felo=felodipine; Solv =solvent; Aldo=aldosterone; Dext= dextrose. P-values on the right: comparison of mean absolute changes from baseline during the last three clearance periods.

red to baseline, Fig. 3).

Infusion of aldosterone alone induced sodium and chloride retention (Table 2 and Fig. 2). Probably due to a steadily decreasing potassium excretion before aldosterone infusion, FE_K during aldosterone infusion did not differ from baseline level. However, as can be seen in Fig. 2, FE_K gradually increased above the level measured just before starting aldosterone infusion ($P<0.01$). FDR_{Na} and $U_K/U_{(Na+K)}$ were increased by aldosterone, whereas FPR_{Na} was not changed (Fig. 3).

Adding aldosterone to the ongoing felodipine infusion attenuated the natriuretic effect of felodipine and counteracted the felodipine-mediated decreases of $U_K/U_{(Na+K)}$ and FDR_{Na} (Fig. 2 and 3). However, $U_{Na}V$ during simultaneous infusion of felodipine and aldosterone clearly remained above baseline level (261 ± 34 and 158 ± 24 μmol/min, $P<0.01$, Table 2). Compared to felodipine alone, combined infusion of felodipine and aldosterone induced a sharp increase in FE_K, which clearly exceeded the kaliuretic effect of aldosterone alone ($+42\pm12$ and $+7\pm8\%$ compared to baseline, $P<0.02$, Fig. 2). As expected, aldosterone had no effect on the felodipine-mediated increase of diuresis and decrease of FPR_{Na} (Fig. 2 and 3).

On all three study days, similar small but significant (all $P<0.02$ compared to baseline) decreases of serum potassium were observed (-0.3 ± 0.1 with felodipine alone, -0.2 ± 0.1 with aldosterone alone, and -0.3 ± 0.1 mmol/L with the combination, $P=0.94$).

Table 2. *Electrolyte excretions and lithium clearances.*

Infusion:	Aldo	Felo+Aldo	Felo	*P* values*	
				A	*B*
$U_{Na}V$ *(μmol/min)*					
baseline	156±24	158±24	138±19		
infusion	64±9†	261±34†	435±70†	<0.001	<0.01
U_KV *(μmol/min)*					
baseline	89±7	87±10	75±10		
infusion	88±8	114±8‡	66±8	0.01	<0.01
$U_{Cl}V$ *(μmol/min)*					
baseline	131±21	126±19	102±13		
infusion	56±8†	236±30†	312±53†	<0.001	0.03
Cl_{Li} *(ml/min)*					
baseline	29±2	31±2	29±1		
infusion	30±1	44±3†	40±3†	<0.001	0.76

Values are means±SEM. Felo=felodipine; Aldo=aldosterone.

**P* values: comparison of absolute changes from baseline induced by infusion; *P* value A: felo+aldo vs aldo alone; *P* value B: felo+aldo vs felo alone.

† $P < 0.01$ and ‡ $P < 0.02$ compared to baseline.

Hormones and plasma drug levels

PRA and PAC were measured for the first time 40 minutes after the start of felodipine or solvent, i.e. just before starting aldosterone or dextrose. These values as well as the means of the three values obtained thereafter are presented in Figure 4. Prior to the start of aldosterone, felodipine had resulted in a higher PRA than solvent, but this rise in PRA was not accompanied by a rise in PAC. Consequently, the PAC/PRA ratio was significantly lower 40 minutes after start of felodipine than after solvent (0.33±0.07 and 0.61±0.13, $P<0.03$). During continued infusion of felodipine alone, PRA did not change (Δ PRA -15±9 %, $P=0.15$), whereas PAC decreased by 26±8 % ($P<0.03$). PAC/PRA ratio remained suppressed (0.31±0.07). Exogenous aldosterone either added to felodipine or solvent increased PAC approximately tenfold, but the levels were somewhat lower during combined infusion with felodipine ($P<0.01$, Fig. 4).

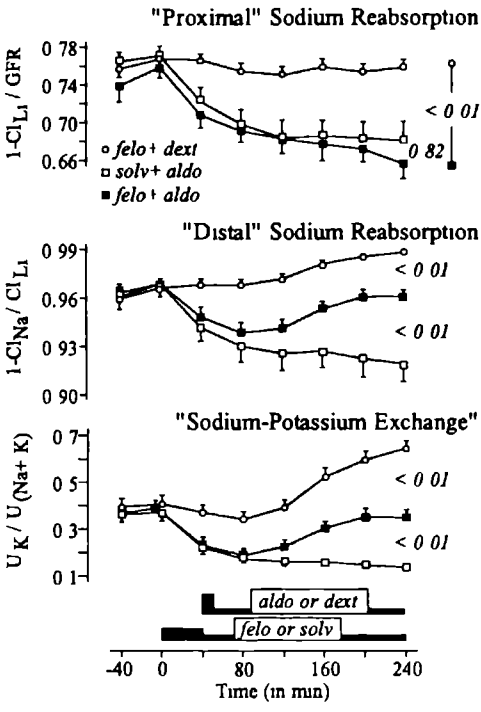

Figure 3. Fractional proximal and distal tubular sodium reabsorption and sodium-potassium exchange in the distal tubule before (baseline) and during drug and placebo infusions. Felo=felodipine; Solv=solvent; Aldo=aldosterone; Dext=dextrose. P-values on the right: comparison of mean absolute changes from baseline during the last three clearance periods.

Plasma felodipine levels were comparable before the start of aldosterone and dextrose (11.2±1.2 and 9.4±1.0 nmol/L, respectively) and remained stable thereafter without being influenced by simultaneous aldosterone infusion (10.4±0.4 compared to 10.3±0.5 nmol/L with felodipine alone). Felodipine plasma levels were within the therapeutic range (4-15 nmol/L)[26].

DISCUSSION

This study in healthy volunteers deals with the effects of exogenous aldosterone added to an ongoing felodipine infusion. As in our previous studies[10,15], felodipine infusion had a distinct natriuretic and diuretic effect without increasing kaliuresis. Although infusion of calcium entry blockers into the renal artery may lead to some kaliuresis in animals[27,28], in most clearance studies in humans felodipine and related dihydropyridine calcium entry blockers fail to increase urinary potassium excretion despite a clearcut rise in sodium excretion[2,12-14]. This suggests that calcium entry blockers somehow act on the distal tubule. Theoretically, calcium entry blockers could inhibit potassium secretion by a direct effect on electrolyte transport at these distal tubular sites. Alternatively, they could specifically counteract the local action of aldosterone, or they could inhibit aldosterone release by the adrenal glands. In the present study, administration of exogenous aldosterone reduced sodium excretion and increased potassium excretion, especially when it was added to the ongoing felodipine infusion. Although the PACs during infusion were in the high physiological range[29,30], our results demonstrate that felodipine

is certainly not capable of fully blocking the effects of aldosterone. Moreover, the fact that aldosterone induced a larger kaliuresis during combined infusion with felodipine despite a somewhat lower PAC could suggest that the effect of aldosterone was even more pronounced during felodipine. However, the effects of aldosterone on FDR_{Na} and $U_K/U_{(Na+K)}$ during felodipine and solvent are difficult to compare because the felodipine-induced changes could have influenced the effects of subsequent aldosterone infusion. Therefore, we cannot exclude with certainty that felodipine has any inhibitory effect on the local action of aldosterone (vide infra).

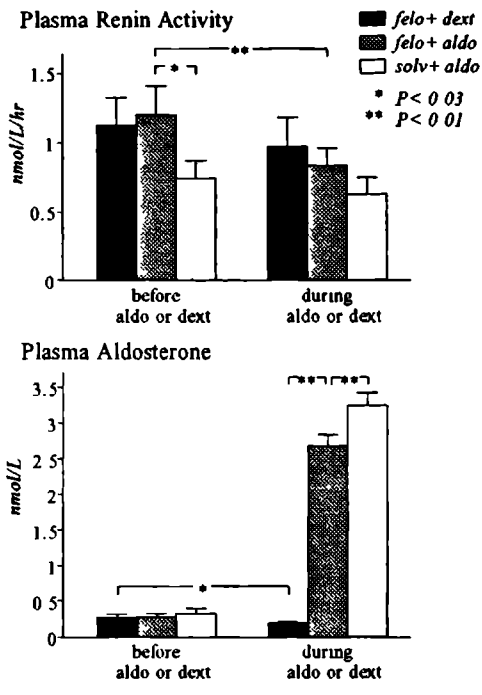

Figure 4. Plasma renin activity (PRA) and plasma aldosterone concentration (PAC) before and during aldosterone (aldo) or dextrose (dext) infusion. Values measured before aldo or dext were determined 40 minutes after starting felodipine or solvent and indicate the acute effect of felodipine on PRA and PAC. The approximately tenfold increases in PAC during both infusions of exogenous aldosterone were also significant compared to values before aldo infusion (not indicated in the Figure)

Our data indicate that calcium entry blockers induce kaliuresis if sufficient amounts of aldosterone are present during treatment. In this respect, it is of interest that felodipine induced a rise of PRA without a concomitant increase of PAC, a dissociation that has been reported frequently[2,17]. Such a rise in PRA is observed only with dihydropyridine calcium entry blockers and is most likely due to sympathetic stimulation and/or a decrease in renal perfusion pressure secondary to blood pressure reduction[27]. The absence of a concomitant rise in PAC could be due to inhibition of aldosterone secretion. In isolated bovine and human adrenal glomerulosa cells, calcium entry blockers and calmodulin inhibitors attenuate the aldosterone release in response to both ANG II and potassium[31-33]. However, although nanomolar concentrations of calcium entry blockers are sufficient to inhibit the effect of potassium[32,34], micromolar concentrations are necessary to attenuate ANG II mediated aldoste-

rone release, possibly because T-type calcium channels are involved in this latter response[35]. In humans, calcium entry blockers blunted ANG II mediated aldosterone release in some[15,36-38], but certainly not all studies[11,39-41]. Therefore, in humans it is uncertain whether the dissociation between PRA and aldosterone is due to direct inhibition of aldosterone release by glomerulosa cells. Alternatively, this dissociation could be the consequence of either increased renal[42] or hepatic clearance of aldosterone secondary to increases in renal and hepatic blood flow[43] induced by calcium entry blockade. Of note, PACs during combined infusion of felodipine and aldosterone were lower than during aldosterone alone, possibly due to such an increased hepatic or renal clearance. Regardless of the mechanism actually involved, our data suggest that calcium entry blockers fail to induce excess urinary potassium loss at least partly because of their intrinsic ability to prevent a rise in PAC.

The effects of exogenous aldosterone on potassium excretion were more pronounced during concomitant infusion of felodipine. At a fixed plasma aldosterone level, potassium excretion will depend on the electrochemical gradient across the apical tubular membrane in the cortical collecting ducts[44] and thus on both distal tubular sodium reabsorption as well as distal tubular flow rate[16]. Therefore, the difference in kaliuretic effect of aldosterone during felodipine and solvent is most readily explained by a felodipine-mediated increase in sodium and fluid delivery to distal tubular sites. It thus supports a proximal tubular site of action of calcium entry blockers, as already suggested by micropuncture studies in animals[3-5] and by clearance studies in humans[9,11-13].

In view of such an effect of calcium entry blockers on proximal tubular sodium reabsorption, the question remains why the resulting increase of distal tubular sodium and fluid load does not lead to increased potassium excretion when these drugs are given alone, because calcium entry blockers do not actually decrease PAC in placebo controlled studies[2,11,14,15,18,42]. The fall of PAC that we observed during felodipine is not specific for this drug, because a similar decrease has been observed during placebo infusion (unpublished observations). However, it is possible that our methods are not sensitive enough to detect small but physiologically important decreases of PAC. Alternatively, calcium entry blockers could still have some inhibitory effect on the local action of aldosterone or could decrease distal tubular sodium reabsorption by some other mechanism. However, increasing PAC can apparently overrule possible other effects of calcium entry blockade on distal tubular sodium reabsorption and the ability of these drugs to prevent a rise of PAC seems essential for the absence of increased kaliuresis.

Some factors might have confounded the results of our study. First, 24-hour sodium excretion was lower before felodipine alone, suggesting a somewhat lower sodium intake in this period, which could have blunted the natriuretic effect on felodipine alone. However, during combined infusion of felodipine and aldosterone natriuresis was still much lower than during felodipine alone, thereby giving even more support to our conclusions. Second, during combined infusion with felodipine and aldosterone, a slight but significant increase in GFR was observed. We cannot exclude that this rise in GFR, although it did not differ from the change observed during felodipine alone, has contributed to an increased tubular sodium and fluid load and has favoured potassium secretion in this period. Third, it could be questioned whether physiologic amounts of aldosterone were administered. The infusion rate we used (0.83 μg/min = 1200 μg/day) approximately equals the maximum aldosterone secretion rate in healthy volunteers[30]. Endogenous aldosterone levels as high as the values that we observed during the infusion of exogenous aldosterone have been reached in ambulatory subjects only by combining a sodium restricted diet with high potassium intake or with diuretics[29,30]. Thus, our results should be confirmed in future studies using a model with a modest increase of endogenous aldosterone prior to infusion of felodipine.

In conclusion, administration of exogenous aldosterone during felodipine attenuates its natriuretic effect and leads to a more pronounced kaliuresis than during aldosterone alone. Our data suggest that felodipine-mediated inhibition of aldosterone release plays an essential role in prevention of increased potassium excretion and also contributes to the natriuretic effect of this type of drug. Moreover, the pronounced kaliuresis during combined infusion of felodipine and aldosterone indicates increased distal delivery of sodium by felodipine and thus supports a proximal tubular site of action of felodipine.

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CHAPTER 5

Metoclopramide stimulates kaliuresis during felodipine without affecting its natriuresis

Henk W. van Hamersvelt, Jack F.M. Wetzels, Robert A.P. Koene, and Frans Th.M. Huysmans.

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ABSTRACT

Calcium entry blockers (CEB) such as felodipine induce an acute natriuresis without a parallel rise of potassium excretion. Previous studies with exogenous aldosterone and felodipine have suggested that the absence of kaliuresis might be explained by a CEB-induced inhibition of aldosterone release. The natriuresis with CEB could not be attributed to a similar mechanism, but might be due to stimulation of intrarenal natriuretic systems such as the dopaminergic system. We therefore studied whether the dopamine antagonist metoclopramide could prevent the natriuresis with low and therapeutic doses of felodipine and whether metoclopramide-induced aldosterone release promoted kaliuresis with felodipine.

Twelve healthy male volunteers participated in a randomized, placebo-controlled, crossover study comparing felodipine infusion during metoclopramide (10 mg/hr) with felodipine alone. Metoclopramide *per se* induced a slight renal vasoconstriction. However, metoclopramide had no significant influence on the pronounced and dose-dependent increases of renal plasma flow and urinary sodium excretion with felodipine. Metoclopramide increased plasma aldosterone concentration (PAC) from 0.17 ± 0.03 to 0.60 ± 0.14 nmol/L and subsequent felodipine infusion clearly increased urinary potassium excretion by 23 ± 6 and 35 ± 8 μmol/min (low and therapeutic doses). In contrast, potassium excretion remained stable with felodipine alone ($+5 \pm 4$ and $+7 \pm 5$ μmol/min, both $P < 0.01$ compared to felodipine during metoclopramide).

In conclusion, the natriuretic action of CEB cannot be explained by stimulation of the dopaminergic system. This natriuresis is accompanied by kaliuresis only in the presence of elevated endogenous PACs. The ability of CEB to prevent a rise of PAC thus seems essential for the prevention of urinary potassium losses.

INTRODUCTION

Calcium entry blockers (CEB), especially dihydropyridines such as felodipine (Plendil^{R}), are powerful vasodilating drugs useful for the treatment of hypertension[1]. These drugs have acute diuretic and natriuretic effects[2], which may contribute to their antihypertensive action. Clearance studies in humans[3-6] and some micropuncture studies in animals[7-9] have indicated that CEB decrease proximal tubular sodium reabsorption. However, the mechanisms responsible for CEB-mediated natriuresis remain unknown. Since selective administration of a CEB into the renal artery stimulates natriuresis[10] an interaction with intrarenal natriuretic systems such as the dopaminergic system[11] could be involved.

We and others have observed that the increased sodium excretion during calcium entry blockade is not accompanied by a parallel increase of potassium excretion[2,3,6,12]. This is rather surprising in view of the aforementioned CEB-mediated decrease of proximal tubular sodium reabsorption. We have previously demonstrated that simultaneous administration of exogenous aldosterone and felodipine was followed by a large increase of kaliuresis[13]. Therefore, the attenuation of potassium excretion during CEB might be explained by the well-known CEB-mediated inhibition of aldosterone release[14].

Dopamine exerts its biological effects through occupation of the DA_1 and DA_2 receptor subtypes[15]. Stimulation of DA_1 receptors induces renal vasodilation and natriuresis[11,16], whereas activation of the DA_2 receptors leads to inhibition of norepinephrine and of aldosterone release[11,17,18]. Therefore, DA_1 receptor stimulation should be considered as a possible mechanism mediating natriuresis with CEB, especially since DA_1 receptor blockade attenuated the natriuretic effect of CEB in spontaneously hypertensive rats[19].

Since selective DA_1 receptor antagonists are not available for human studies, we used metoclopramide to delineate the role of the dopaminergic system in the natriuretic effect of different doses of felodipine. Metoclopramide also increases plasma aldosterone concentration (PAC)[20] and thus enabled us to study the relation between CEB-induced natriuresis, endogenous aldosterone, and potassium excretion. In our previous study with exogenous aldosterone, this infusion resulted in relatively high PAC above 2.5 nmol/L. We therefore questioned whether an increase of endogenous aldosterone within the normal physiological range would also increase potassium excretion during CEB-mediated natriuresis.

METHODS

Subjects

Twelve healthy male volunteers participated in this study. All participants were free of medication. Ages ranged from 20 to 33 years (mean 26) and all had a blood pressure below 140/90 (ranges, systolic 102 to 140 and diastolic 66 to 86 mmHg), normal length (range, 172 to 191 cm) and body weight (range, 64 to 81 kg).

The study protocol was approved by the hospital ethics committee and all volunteers gave written informed consent.

Study protocol

The effects of felodipine during metoclopramide were compared to felodipine alone. As control, also placebo infusions were given. Thus, in three clearance experiments separated by washout periods of one week, all subjects received combined intravenous infusions of metoclopramide and felodipine, of placebo (5% dextrose) and felodipine, and of two placebos (5% dextrose and solvent). A randomised, single blind, two-way crossover design was used with the combination of metoclopramide and felodipine in order always immediately following dextrose and felodipine. Randomisation was balanced in blocks of two. Subjects were advised to keep their sodium intake constant on the last three days preceding clearance experiments. Dietary intake was checked by measuring creatinine, sodium and potassium in 24-hour urine samples collected on the last two days preceding clearance studies. On the eve of each clearance study, 600 mg lithium carbonate (16.2 mmol lithium) was given orally. Subjects were asked to refrain from smoking and the use of alcohol for the last 24 hours and from caffeine-containing beverages for the last eight hours preceding clearance studies.

On study days, the subjects consumed a light breakfast and drank 375 ml tap water. One hour thereafter, clearance experiments took place between 9.00 hr and 16.00 hr. Upon arrival, body weight was measured and water diuresis was induced by an additional oral water load of 15 ml/kg body weight, resulting in a urinary osmolality of 79 mosmol/kg (range, 48 to 122). During the whole experiment, 0.25 % sodium chloride in 3.3 % dextrose was infused at a rate of

400 ml/hr to maintain diuresis and to compensate for sodium losses observed previously during similar experiments with placebo[21]. Urinary volume losses in excess of 400 ml/hr were replaced orally by tap water. Subjects remained supine except for spontaneous voiding. With a continuous infusion technique described elsewhere[12] renal plasma flow (RPF) and glomerular filtration rate (GFR) were estimated by measurement of renal clearances of para-aminohippuric acid (PAH) and inulin (polyfructosan, Inutest^{R}, Laevosan-Gesellschaft, Linz, Austria), respectively. After 90 min equilibration, two baseline urine samples of 30 min were collected. Thereafter, metoclopramide (Primperan^{R}, Delagrange, Chilly-Mazarin, France) in 5% dextrose (1 mg of metoclopramide/ml) or 5% dextrose alone was infused through a separate intravenous cannula in the upper arm at an infusion rate of 20 ml/hr for the first 30 min and 10 ml/hr until the end of the experiment (cumulative dose 45 mg metoclopramide in 240 min). During metoclopramide and dextrose, two 30 min urine collections were made before felodipine or solvent was started in order to study the effect of pretreatment *per se*. Based on earlier studies[3], we used a felodipine infusion schedule aiming at stable, subtherapeutic levels for the first 90 min (low dose) and therapeutic levels[22] for the last 90 min (therapeutic dose). To reach this, 0.10 μg/kg/min of felodipine (Plendil^{R}) was infused for the first 30 min, followed by 0.04 μg/kg/min for the following 60 min (cumulative dose 0.39±0.04 mg in 90 min, mean±SD). Thereafter, the infusion rate was increased to 0.14 μg/kg/min for another 30 min and 0.08 μg/kg/min for the last 60 min (cumulative dose 0.66±0.05 mg in the last 90 min). Six additional 30 min urine samples were collected during felodipine and solvent infusions. Blood samples were drawn at the beginning and the end of each urine collection period. In blood and urine samples, PAH, inulin, sodium, potassium and chloride concentrations were measured by standard (semi)automated techniques, whereas lithium was determined by atomic absorption spectrophotometry. In blood samples, hematocrit (Ht) was determined by routine Coulter Counter, plasma renin activity (PRA) and PAC by radioimmunoassay[23,24], and plasma felodipine levels by gas chromatography[25]. Blood pressures and pulse rates were recorded at three min intervals by an automatic device (Dinamap model 1846P, Critikon, Florida, USA), which directly measures mean arterial pressure (MAP). The mean values of five consecutive readings in the middle of each urine collection period were used for analysis.

Of the various substances (x), clearances (Cl_x), urinary excretions (U_xV) and fractional excretions (FE_x) were calculated according to standard formulas[26] (U_x representing the urinary concentration and V the urinary flow rate). Filtration

fraction (FF) was calculated by (GFR/RPF)x100 (%) and renal blood flow (RBF) by RPF/(1-Ht) (ml/min). GFR and RPF were adjusted to a standard body surface area of 1.73 m^2. Renal vascular resistance (RVR) was calculated by 1000xMAP/RBF (arbitrary units). Fractional proximal sodium reabsorption (FPR_{Na}) and fractional distal sodium reabsorption (FDR_{Na}) were calculated by the lithium clearance method[27]:

$FPR_{Na} = (1\text{-}Cl_{Li}/GFR)x100$ (%).

$FDR_{Na} = (1\text{-}Cl_{Na}/Cl_{Li})x100$ (%).

Statistical analysis

For baseline levels, means of the first two urine collection periods were calculated. The fourth urine collection period, measured just before starting felodipine or solvent, was used as pretreatment level. For the low and the therapeutic felodipine doses, mean values of the last two urine collections of each 90 min period were calculated, i.e. mean values of the sixth and seventh and mean values of the ninth and tenth clearance period were used, respectively. The effects of metoclopramide pretreatment *per se* were evaluated by comparing absolute changes from baseline level. The responses to felodipine infusions were evaluated by comparing absolute changes from pretreatment levels as measured just before starting felodipine or solvent. Infusion of felodipine alone was compared on the one hand to placebo infusion and on the other hand to felodipine during metoclopramide.

Statistics were performed with SAS (Statistical Analysis System) software, using two tailed Wilcoxon's test for simple pairwise comparisons and repeated measures analysis of variance (ANOVA) for comparison of baseline data. Probability values below 0.05 were considered statistically significant. Results are presented as means$\pm$SEM.

RESULTS

All 12 volunteers completed the three clearance experiments. The most common side effects were restlessness during or after metoclopramide infusion (6/12) and self-limiting headache during felodipine infusion (4/12 compared to 2/12 with solvent). One volunteer complained of drowsiness during felodipine infusion.

Table 1. *Steady state data before clearance studies*

	Dext+Solv	Dext+Felo	Meto+Felo
Body weight *(kg)*	73.1±1.5	72.9±1.6	72.9±1.6
Urinary volume *(ml/24 hr)*	1315±83	1488±104	1424±134
Urinary sodium *(mmol/24 hr)*	135±10	139±9	156±20
Urinary potassium *(mmol/24 hr)*	75±5	77±7	80±8

Values are means±SEM. Dext=dextrose used as placebo; Solv=solvent used as placebo; Felo=felodipine; Meto=metoclopramide.

Table 2. *Baseline values of electrolyte excretions and systemic and renal hemodynamics*

	Dext+Solv	Dext+Felo	Meto+Felo	P-values*
MAP *(mm Hg)*	91±2	91±3	88±2†	0.02
Heart rate *(beats/min)*	58±2	55±2	54±2	0.18
RPF *(ml/min/1.73 m²)*	641±30	632±39	586±31	0.10
GFR *(ml/min/1.73 m²)*	126±5	125±4	122±6	0.69
FF *(%)*	20±1	21±1	22±2	0.52
Urinary flow *(ml/min)*	15±1	15±1	16±1	0.56
FE_{Na} *(%)*	0.9±0.1	0.8±0.1	1.0±0.2	0.25
FE_{K} *(%)*	17±2	13±2	16±2	0.08
FE_{Cl} *(%)*	1.0±0.2	0.9±0.1	1.1±0.2	0.25
FDR_{Na} *(%)*	97±0	97±0	96±1	0.30
PRA *(nmol/L/hr)*	0.66±0.13	0.79±0.07	0.76±0.14	0.61
PAC *(nmol/L)*	0.34±0.15	0.28±0.09	0.17±0.03	0.32

Values are means±SEM. Baseline values were measured before starting drugs.

Dext=dextrose used as placebo; Solv=solvent used as placebo; Felo=felodipine; Meto=metoclopramide. For other abbreviations see text.

* Overall P-values for comparison of the three treatment periods by repeated measures analysis of variance. † $P < 0.05$ compared to dext+felo.

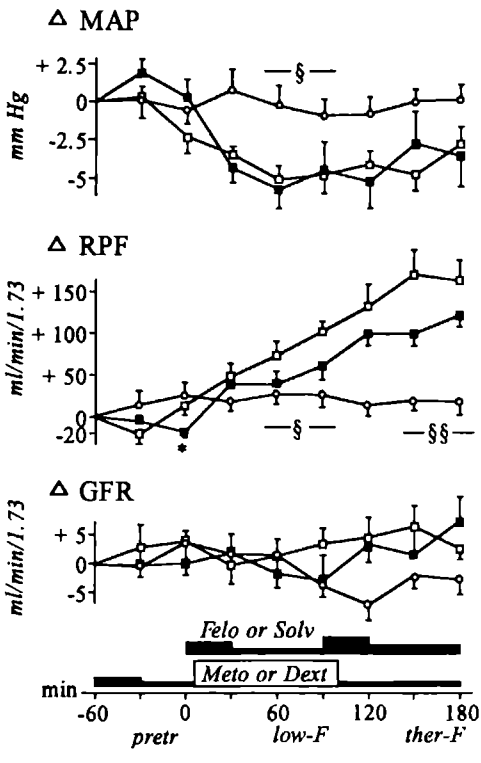

*Figure 1. Changes of sytemic and renal hemodynamics. (○) =dextrose (dext) followed by solvent (solv); (□) =dextrose followed by felodipine (felo); (■) =metoclopramide (meto) followed by felodipine. Pretr=pretreatment; Low-F and ther-F= low and therapeutic doses of felodipine; *$P<0.05$ for comparison of dext+felo to meto+felo (changes from baseline); §$P<0.05$ and §§$P<0.01$ for comparison of dext+felo to dext+solv (changes from pretreatment).*

Baseline data

Body weights and 24-hour urinary water, sodium, and potassium excretions were comparable before the three clearance experiments (Table 1). Baseline electrolyte excretions, and baseline levels of systemic and renal hemodynamics did not differ between the three experimental periods except for the baseline MAP, which was slightly lower preceding metoclopramide infusion (Table 2).

Systemic and renal hemodynamics

Infusion of low dose felodipine decreased MAP from a pretreatment level of 89±3 to 86±2 mmHg (Fig. 1) and increased heart rate from 55±2 to 60±2 beats/min (both $P<0.05$ compared to changes with solvent). Therapeutic dose felodipine increased heart rate further to 65±3 beats/min ($P<0.01$), whereas the change of MAP no longer differed from the change with solvent ($P>0.10$, Fig. 1). Metoclopramide pretreatment did not change blood pressure or heart rate and did not influence the felodipine-mediated blood pressure changes. During simultaneous infusion of metoclopramide and therapeutic dose felodipine, the rise of heart rate was more pronounced than with felodipine alone (+16±3 and +10±1 beats/min, respectively, $P<0.05$).

Felodipine infusion alone induced a dose related increase of RPF (Fig. 1) and a decrease of FF (-2.3±0.4 and -3.7±0.5 % at low and therapeutic dose, both $P<0.05$ compared to solvent). Felodipine did not change GFR (Fig. 1).

Metoclopramide infusion *per se* had no influence on GFR and FF, but induced a slight renal vasoconstriction. RPF decreased from 586±31 to 568±29 ml/min/1.73 m² (Δ RPF -18±7 compared to +14±11 ml/min/1.73 m² with dextrose, $P<0.02$, Fig. 1) and RVR increased from 91±6 to 94±5 units (Δ RVR +3±2 compared to -3±2 units with dextrose, $P<0.02$). However, felodipine infusion induced the same renal vasodilation during metoclopramide as during dextrose with comparable decreases of RVR (data not shown) and increases of RPF (low dose +68±14 and +74±16 ml/min/1.73 m²; therapeutic dose +128±15 and +152±27, $P>0.10$ for both doses, Fig. 1).

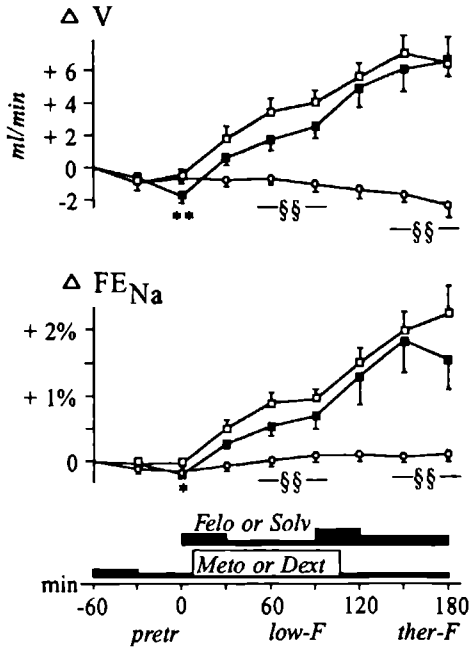

*Figure 2. Changes of urinary flow rate (V) and fractional sodium excretion (FE_{Na}); (○) =dextrose followed by solvent; (□) =dextrose followed by felodipine; (■) =metoclopramide followed by felodipine. See Figure 1 for abbreviations. *$P<0.05$ and **$P<0.01$ for comparison of dext+felo to meto+felo (changes from baseline); §§$P<0.01$ for comparison of dext+felo to dext+solv (changes from pretreatment).*

Diuresis and natriuresis

Felodipine infusion alone had a distinct and dose-dependent diuretic and natriuretic effect with parallel changes of chloride excretion (Fig. 2 and Table 3). Metoclopramide *per se* induced a decrease of urinary flow rate and a minor decrease of sodium excretion which was only significant compared to dextrose preceding felodipine (Table 3 and Fig. 2). During ongoing metoclopramide infusion, felodipine still induced pronounced increases of natriuresis and diuresis, which were comparable to the responses with felodipine alone. Only the increase of $U_{Na}V$ induced by low dose felodipine tended to be attenuated by metoclopramide (+136±29 μmol/min compared to +171±26 with felodipine alone, $P=0.06$).

Both doses of felodipine significantly increased Cl_{Li} (Table 3) and decreased

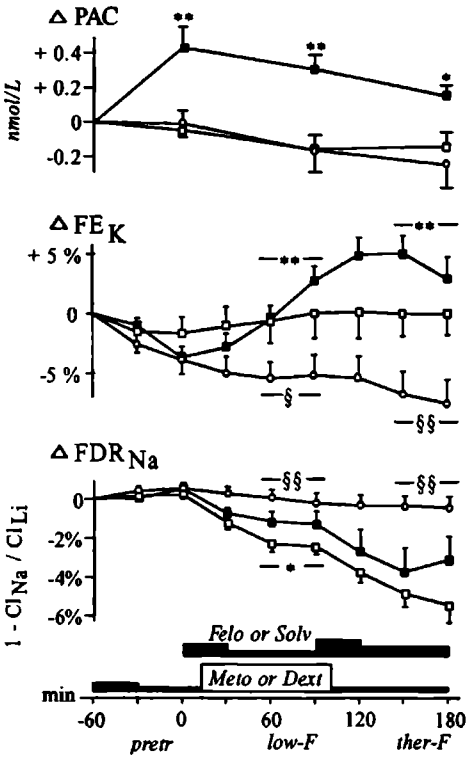

Figure 3. Changes of plasma aldosteron e concentration (PAC), fractional potassium excretion (FE_K) and calculate d distal sodium reabsorption (FDR_{Na}); (○) =dextrose followed by solvent; (□) =dextrose followed by felodipine; (■) =metoclopramide followed by felodipine. See Figure 1 for abbreviation s. $^{*}P<0.05$ and $^{**}P<0.01$ for comparison of dext+felo to meto+felo (changes from baseline); $^{\S}P<0.05$ and $^{\S\S}P<0.01$ for comparison of dext+felo to dext+solv (changes from pretreatment).

calculated FPR_{Na} (data not shown) and FDR_{Na} (Fig. 3). Metoclopramide *per se* induced a slight decrease of Cl_{Li} (Table 3) and tended to increase calculated FPR_{Na} ($P=0.08$ compared to dextrose preceding felodipine). However, metoclopramide did not affect the large felodipine-mediated increase of Cl_{Li} and decrease of FPR_{Na}. Only the decrease of FDR_{Na} induced by low dose felodipine was blunted by metoclopramide (-1.8 ± 0.5 compared to -2.6 ± 0.3 % with felodipine alone, $P<0.05$).

Kaliuresis

On placebo days, potassium excretion steadily decreased with time (Fig. 3 and Table 3). In contrast, no such decrease of kaliuresis was observed with felodipine alone, thus suggesting that felodipine caused a slight relative increase of potassium excretion. Metoclopramide *per se* had no significant effect on potassium excretion, but adding felodipine to metoclopramide resulted in a prompt and clearcut increase of kaliuresis (Table 3 and Fig. 3). FE_K thus increased from 13 ± 2 % to 18 ± 2 at low and 20 ± 2 % at therapeutic dose felodipine (Δ FE_K $+5\pm1$ % and $+8\pm2$ % compared to $+1\pm1$ and $+2\pm1$ % with felodipine alone, both $P<0.01$).

Hormones and plasma drug levels

PRA gradually decreased during combined placebo infusion from a baseline level of 0.66±0.13 to 0.46±0.14 nmol/L/hr at the end of the experiment. On the other two experimental days, similar decreases of PRA were observed without any significant influence of metoclopramide nor of either dose of felodipine. PRA decreased by 0.20±0.08 nmol/L/hr with felodipine alone and by 0.07±0.11 with felodipine during metoclopramide.

PAC also steadily decreased on placebo days and with felodipine alone

Table 3. *Electrolyte excretions before and during drug and placebo infusions.*

		Baseline	Before F	Low-F	Ther-F
$U_{Na}V$ *(μmol/min)*	Dext+Solv	155±20	134±14	167±20 *	175±19 *
	Dext+Felo	142±17	141±21 *	312±37	537±65
	Meto+Felo	171±27	139±22	275±38	489±81
$U_{K}V$ *(μmol/min)*	Dext+Solv	91±9	70±4 †	58±4 *	50±4 *
	Dext+Felo	70±8	63±7	68±8 †	70±7 *
	Meto+Felo	84±11	66±9	88±12	100±10
$U_{Cl}V$ *(μmol/min)*	Dext+Solv	129±18	100±11	110±16 *	101±13 *
	Dext+Felo	115±16	98±14	226±24	423±54
	Meto+Felo	143±23	114±16	239±35	430±71
Cl_L *(ml/min)*	Dext+Solv	32±2	31±2	31±2 *	31±2 *
	Dext+Felo	31±2	31±2 †	38±2	43±2
	Meto+Felo	29±2	27±2	36±3	44±4

Values are means±SEM. Baseline values were measured before starting drugs.

Before F=before starting felodipine or solvent, effect of pretreatment; low-F and ther-F=low and therapeutic doses of felodipine; Dext=dextrose used as placebo; Solv=solvent used as placebo; Felo=felodipine; Meto=metoclopramide. For other abbreviations see text.

Baseline values were comparable (ANOVA). * $P < 0.01$ and † $P < 0.05$ compared to dext+felo, comparison of absolute changes from baseline level for pretreatment effect and absolute changes from pretreatment level for responses to felodipine infusion.

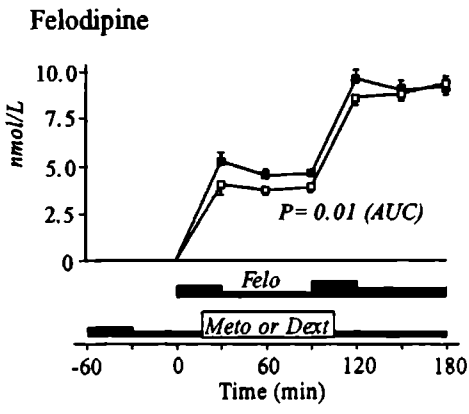

Figure 4. Plasma felodipine (felo) levels during concomitant dextrose (dext, □) and metoclopramide (meto, ■) infusion. P value for comparison of area under the curves (AUC).

(Fig. 3). However, metoclopramide *per se* induced a large rise of PAC from 0.17±0.03 to 0.60±0.14 nmol/L ($P<0.01$ compared to dextrose) and PAC remained clearly higher during subsequent felodipine infusion than during felodipine alone (low dose 0.47±0.10 vs 0.12±0.02 nmol/L, $P<0.001$ and therapeutic dose 0.32±0.08 vs 0.13±0.04, $P=0.02$, Fig. 3).

Infusion of low dose felodipine led to stable, subtherapeutic plasma levels of approximately 4 nmol/L. Increasing the dose more than doubled the drug levels to a therapeutic level[22] of 9 nmol/L (Fig. 4). Felodipine plasma levels were slightly but significantly higher during concomitant metoclopramide infusion (low dose 4.8±0.3 compared to 3.9±0.3 nmol/L with felodipine alone; therapeutic dose 9.3±0.4 vs 9.0±0.3, $P<0.05$ for area under curve).

DISCUSSION

In the present study in healthy volunteers, the aselective dopamine antagonist metoclopramide had no significant influence on the natriuretic and diuretic effect of either the low or the therapeutic dose of felodipine. Only the natriuretic effect of low dose felodipine was slightly, but insignificantly attenuated by metoclopramide. Thus, our data suggest that the natriuretic and diuretic effects of CEB cannot be explained by an interaction with the intrarenal dopaminergic system.

Metoclopramide exhibits a preferential DA_2 receptor selectivity[28,29], however, at high dose also DA_1 receptors are blocked[30]. Thus, the question arises whether the dose in our study was high enough to block the tubular DA_1 receptors, which mediate dopamine-induced natriuresis[11,16,31]. Studies in healthy volunteers have demonstrated that the same metoclopramide infusion schedule as used in our study inhibited the natriuretic action of a low dose of dopamine[32] and also the natriuretic response to the selective DA_1 agonist fenoldopam[33]. These data

indicate that our metoclopramide dose is indeed capable of blocking DA_1 receptor mediated natriuresis. The use of a selective DA_1 receptor antagonist would of course have allowed more definitive conclusions, but such compounds are not available for human studies.

Our data seem to be in contrast to animal studies, because the selective DA_1 antagonist SCH-23990 prevented the natriuretic effect of nitrendipine and diltiazem in spontaneously hypertensive rats[19]. However, it is difficult to translate these data to the human situation because very high drug doses were used and, consequently, very large changes of blood pressure were observed. Of note, in these rats indomethacin also attenuated the natriuresis with diltiazem[19], whereas such an effect could not be confirmed in humans[34].

Our results do not exclude the possibility that metoclopramide slightly attenuates the natriuretic effect of low doses of CEB. Although the effects of metoclopramide on felodipine-induced natriuresis failed to reach statistical significance, sodium excretion tended to be lower. Plasma felodipine levels were, however, higher during concomitant metoclopramide infusion, possibly due to a decreased metabolic clearance of felodipine. As the natriuretic effects of CEB appear to be dose dependent[35], one might have expected higher rates of sodium excretion in this period with higher felodipine levels. Our data are thus in line with observations in hypertensive patients, where metoclopramide largely prevented the natriuresis of a very low dose of nicardipine, but did not affect the natriuretic action of a therapeutic dose[36]. Taken together, these data might suggest a, albeit limited, role for the dopaminergic system in CEB-induced natriuresis. However, the attenuation of the natriuretic effect of low doses of CEB could easily be explained by other effects of metoclopramide, which are not mediated through blockade of DA_1 receptors. Indeed, metoclopramide induced a large increase of PAC, which might have attenuated the natriuresis with felodipine comparable to the attenuation of natriuresis in our study with exogenous aldosterone and felodipine[13]. This possibility is further supported by the results of the present study where metoclopramide attenuated the felodipine-mediated decrease of distal and not of proximal tubular sodium reabsorption.

In the setting of elevated PAC during metoclopramide infusion, felodipine induced an immediate increase of potassium excretion. This observation is noteworthy, since we and others have observed that the natriuresis of CEB is usually not accompanied by an increase of kaliuresis[2,3,6,12]. This absence of kaliuresis is surprising in view of the fact that clearance studies in humans[4-6] and some micropuncture studies in animals[7-9] have indicated that CEB decrease proximal tubular sodium reabsorption. Such a decrease in proximal sodium

reabsorption and the subsequent increase of distal tubular sodium and fluid load should normally enhance potassium excretion, as is observed with diuretics such as furosemide[37]. Thus, it seems likely that CEB also act at a distal tubular site. In this respect, it is of interest that dihydropyridine CEB can induce a rise of PRA without a parallel increase of PAC[2,14], possibly due to direct inhibition of aldosterone release by adrenal glomerulosa cells[38,39]. We suggest that this inhibition of aldosterone release is essential for the dissociation between the natriuretic and kaliuretic effect of CEB. In a previous study, we demonstrated that adding exogenous aldosterone to a felodipine infusion resulted in a major increase of potassium excretion that was more pronounced than with aldosterone alone. However, definite conclusions could not be drawn since PAC were relatively high (> 2.5 nmol/L). Our present findings now confirm that elevated endogenous aldosterone levels within the physiological range, as induced by metoclopramide, also enabled felodipine to induce kaliuresis. It should be noted that it is unlikely that metoclopramide administration *per se* is the cause of the increased potassium excretion, because others have not observed such kaliuretic effects of metoclopramide[40-42].

In conclusion, dopaminergic receptor blockade with metoclopramide does not prevent the natriuretic effect of low and therapeutic doses of felodipine, thereby indicating that the dopaminergic system does not play a major role in the natriuretic action of CEB. In contrast to felodipine alone, simultaneous infusion of metoclopramide and felodipine led to a marked kaliuresis as a consequence of the metoclopramide-mediated rise of aldosterone. This confirms earlier findings with exogenous aldosterone and indicates that CEB will induce kaliuresis if sufficient amounts of aldosterone are available. The inhibitory effect of CEB on aldosterone release thus prevents urinary potassium losses despite an increase of natriuresis and diuresis.

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CHAPTER 6

Acute renal effects of felodipine in hypertensive patients with kidney disease

Frans Th.M. Huysmans, Henk W. van Hamersvelt, Henk E. Sluiter, Jack F.M. Wetzels, and Robert A.P. Koene.

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ABSTRACT

In contrast to other types of directly acting vasodilators, calcium entry blockers promote sodium excretion. It is not well established, however, whether these drugs also induce natriuresis in hypertensive patients with renal disease.

Therefore, we studied the acute effects of the dihydropyridine calcium entry blocker felodipine in nine such patients (C_{Cr} 68 ± 19 ml/min) and 12 healthy normotensive subjects. In both the hypertensive patients and the normotensive subjects total and fractional sodium excretion rose during the first 40 minutes of intravenous felodipine infusion: in the hypertensive patients this rise of sodium excretion was positively correlated to the initial glomerular filtration rate (GFR) ($r=0.87$, $P<0.01$). In the patients, during ongoing felodipine infusion, natriuresis was attenuated in the setting of a large continuing decrease of blood pressure. In contrast, in the normotensive subjects, in whom blood pressure did not fall any further, a steady rise of sodium excretion was observed. In both the hypertensive patients and the normotensive subjects GFR remained unchanged and renal vascular resistance decreased, whereas renal plasma flow increased only in the latter group. Changes in sodium excretion were not correlated to changes in renal hemodynamic parameters.

It is concluded, that also in hypertensive patients with diminished renal function felodipine exerts a potentially advantageous natriuretic effect. However, this natriuretic effect is possibly less at lower GFR and seems to be attenuated by blood pressure reduction. The mechanism of this natriuretic effect as well as its contribution to the antihypertensive effect of felodipine still has to be clarified.

INTRODUCTION

Calcium entry blockers are effective antihypertensive agents with few side effects[1]. In contrast to other vasodilators[2,3], they have a diuretic and natriuretic effect in both healthy volunteers and patients with essential hypertension[4-6]. It has been suggested that this natriuretic effect contributes to the antihypertensive effects of these drugs[4,7]. It is not well established, whether calcium entry blockers also induce natriuresis in hypertensive patients with renal disease. In such patients a natriuretic effect of a vasodilator might be of specific value, since in many of them sodium and water retention is directly involved in inducing and sustaining hypertension[2]. Therefore, we studied the influence of the dihydropyridine calcium entry blocker felodipine on sodium excretion, blood pressure, and renal hemodynamics in hypertensive patients with renal disease.

PATIENTS AND METHODS

We studied the acute effects of an intravenous felodipine infusion in nine patients, five men and four women, mean age ($\pm$SD) 40$\pm$14 years, and endogenous creatinine clearance (C_{Cr}) 68$\pm$19 ml/min (expressed per 1.73 m^2 BSA, like glomerular filtration rate, GFR and renal plasma flow, RPF). Three patients had essential hypertension and a C_{Cr} below 100 ml/min and six had hypertension complicating renal disease (three chronic glomerulonephritis, two chronic pyelonephritis, and one polycystic kidney disease). Antihypertensive drugs were discontinued for at least two days. After an oral water load of 800 ml they received 400 ml of fluid and 17 mmol sodium chloride per hour intravenously during five hours; urinary volume losses in excess of the infusion rate were compensated for by oral water intake. After 2.5 hours of equilibration felodipine was infused at a constant rate of 0.01 mg/min for two hours (total dose 1.2 mg). Blood pressure was recorded automatically (Arteriosonde 1225) every two min. GFR and RPF were estimated at 40-minute intervals by measuring the renal clearance of continuously infused inulin (laevofructosan,Inutest^R) and PAH as described previously[8]; in the same periods renal clearances of sodium were determined. During felodipine infusion blood samples were taken for the determination of plasma drug levels by gas chromatography[9]. For comparison, using the same protocol we also studied the effects of felodipine infusion in 12 healthy male volunteers,

age 28±4 years, C_{Cr} 134±12 ml/min. However, they received felodipine at a rate of 0.012 mg/min during the first 40 min, and at a rate of 0.005 mg/min thereafter, resulting in a mean total dose of 0.088 mg.

Mean arterial blood pressure (MAP) was defined as the diastolic plus one third of the pulse pressure. Filtration fraction (FF) was calculated as GFR/RPF. Renal vascular resistance (RVR) was calculated as 1000*MAP/RPF, expressed in arbitrary units. Because of the wide range of RVR values a logarithmic scale was used for graphical presentation. FE_{Na} was expressed as a percentage of GFR.

For statistical analysis of changes of renal clearances and hemodynamic parameters mean values of the last two 40-minute periods preceding felodipine infusion were used as baseline levels. Relative changes from these baseline levels in the three 40-minute periods during felodipine infusion were analyzed by Wilcoxon's rank sum test for paired observations. Correlations were calculated according to Spearman. A P-value of 0.05 was considered as the level of statistical significance. All results are given as means±SEM. The study protocols were approved by the Ethics Committee of the University Hospital Nijmegen.

RESULTS

The influence of felodipine infusion on urinary sodium excretion and on renal and systemic hemodynamics is shown in Figures 1 and 2. In the hypertensive patients absolute and fractional sodium excretions during felodipine infusion were 93±54 % and 100±54 % above pretreatment level, respectively. The mean level of MAP throughout the whole period of felodipine infusion was 12±1 % ($P<0.01$) below and that of heart rate 18±5 % ($P<0.01$) above baseline level. In the last period of felodipine infusion blood pressure had decreased by 17±2 % from an initial level of 171±11/110±4 to 145±9/86±3 mmHg. GFR and RPF did not change significantly. The mean decrease of FF was 10±3 % ($P<0.02$) and that of RVR 15±4 % ($P<0.01$). In the last two periods of felodipine infusion urinary sodium excretion did not rise further, whereas a steady decrease of blood pressure and RVR was observed (Fig. 1 and 2). Plasma felodipine levels increased gradually in each patient during infusion. The correlation between basal GFR and the increase of sodium excretion throughout the whole period of felodipine infusion failed to be significant ($r=0.53$). How-

ever, as shown in Figure 3, the rise of sodium excretion in the first 40 min of felodipine infusion was positively correlated to the initial GFR level (r=0.87, P<0.01). We found no significant correlations between changes in total or fractional sodium excretion on the one hand and changes in systemic or renal hemodynamics on the other hand.

In the healthy volunteers absolute and fractional sodium excretions were 169±28 % (P<0.01) and 161±25 % (P<0.01) above baseline level, respectively. The difference in sodium excretion between normotensive and hypertensive subjects may seem more striking as shown in Figure 1 than as suggested by the percentage changes; however, it should be realized, that graphically the means of absolute values of sodium excretion instead of relative changes are presented. Heart rate was 15±2 % above (P<0.01) and MAP only 5±1 % (P<0.01) below pretreatment level. Blood pressure decreased from an initial level of 121±3/74±3 to 119±3/70±3 mmHg at the end of felodipine infusion. Also here, GFR did not change. During the whole period of felodipine infusion RPF rose by 13±4 % (P<0.02), accompanied by decreases of FF (-8±3 %, P<0.02) and RVR (-14±3 %, P<0.01). Figure 1 shows that in the healthy volunteers sodium excretion rose continuously during felodipine infusion. This steady rise coincided

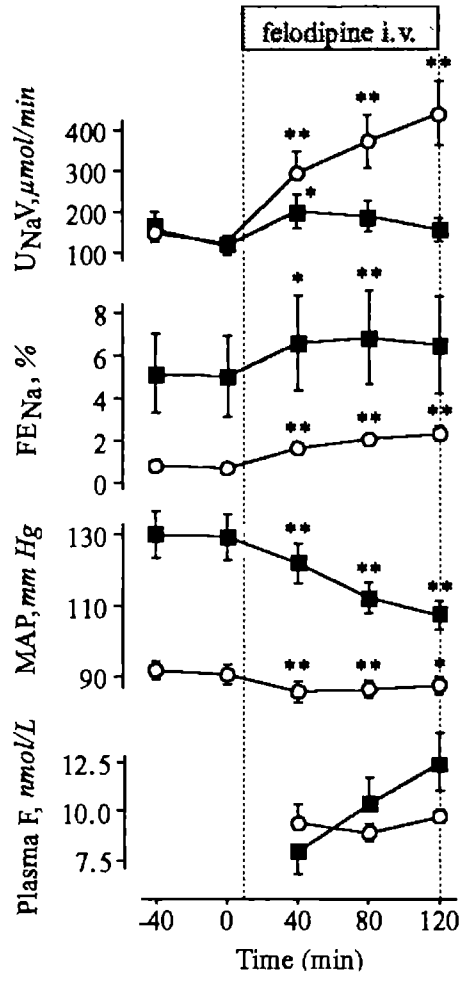

*Figure 1. Total ($U_{Na}V$) and fractional (FE_{Na}) sodium excretion, mean arterial pressure (MAP), and plasma felodipine levels (plasma F) in 12 normotensive volunteers (○) and nine hypertensive patients with renal disease (■) before and during felodipine infusion. *P<0.05 and **P<0.01, compared to baseline values.*

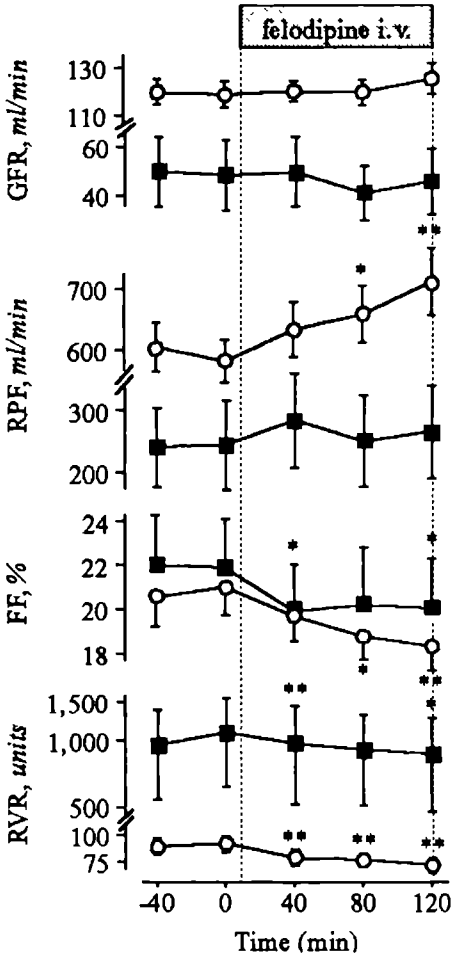

*Figure 2. GFR, RPF, FF, and RVR in 12 normotensive subjects (○) and nine hypertensive patients with renal disease (■) before and during felodipine infusion. *P<0.05 and **P<0.01, compared to baseline values.*

with an ongoing rise of RPF and a further decrease of RVR, but without a further decrease of blood pressure (Fig. 1 and 2). Also in the healthy volunteers we found no correlations between the rise of sodium excretion and the changes of systemic and renal hemodynamic parameters. Due to the slightly different infusion protocol the plasma felodipine levels were about constant throughout the felodipine infusion period.

DISCUSSION

In contrast to the experience with other types of directly acting vasodilators[2,3] we observed a distinct natriuretic effect of the calcium entry blocker felodipine, also in hypertensive patients with renal disease. It is very improbable that the observed increase of sodium excretion has to be attributed to factors other than the administration of felodipine, since sodium excretion was stable in the last two periods before felodipine infusion and increased already in the first period after the start of felodipine. The increase of sodium excretion on felodipine administration in the normotensive volunteers was of about the same magnitude as in comparable studies[5,8,10,11], where also no large decreases of blood pressure after felodipine administration were observed. In comparison to the effects in the normotensive subjects, the natriuresis on felodipine in the hypertensive patients in the present study was not very large and possibly less at lower levels of GFR,

despite comparable initial sodium excretion rates in patients and volunteers. Moreover, in the hypertensive patients sodium excretion did not gradually increase during ongoing felodipine infusion. Theoretically, the small differences in infusion rates of felodipine between the two groups could be responsible for differences in the course of sodium excretion. However, it is clear from Figure 1, that the absence of a further rise of sodium excretion during ongoing felodipine infusion in the hypertensive patients cannot be ascribed to lower plasma felodipine levels in these patients than in the normotensive subjects. The finding that sodium excretion did not increase further in the hypertensive patients could very well be related to the large decrease of blood pressure and hence of renal perfusion pressure at gradually rising plasma felodipine levels in the hypertensive patients[10,12]. This explanation is in agreement with observations in patients with essential hypertension, where acute felodipine administration induced a somewhat less impressive increase of sodium excretion accompanied by a more distinct decrease of blood pressure than in the normotensive subjects in our study[13,14].

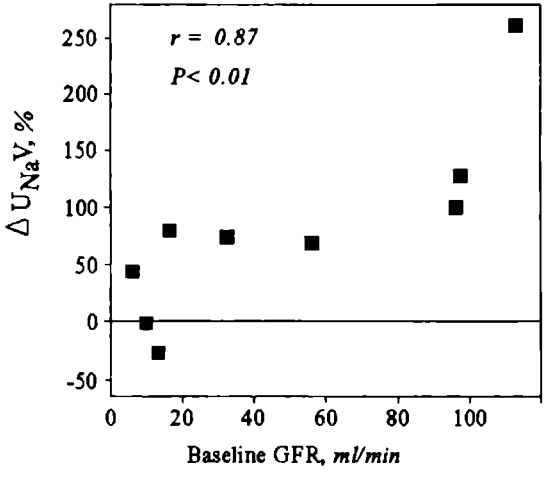

Figure 3. Correlation between baseline GFR and the increase of sodium excretion during the first 40 min of felodipine infusion ($\Delta U_{Na}V$) in nine hypertensive patients with renal disease.

In normotensive subjects and in patients with essential hypertension calcium entry blockers generally induce an acute increase or no change in GFR and RPF and a decrease of RVR, accompanying the rise of sodium excretion[15-18]. After acute administration of felodipine in patients with essential hypertension, rises of renal perfusion without changes in GFR have been observed[13,14]. In the normotensive volunteers we observed a rise of RPF and a decrease of RVR, without a change of GFR, as we have seen before[8]. In accordance with some[19-21], but not all[16] studies concerning the renal effects of calcium entry blockers in patients with kidney disease, we saw no significant increase of RPF, and also no change of GFR in our patients. Yet,

calculated RVR decreased, which may be the consequence of a direct effect of felodipine in the kidney or merely of adaptive autoregulation due to the reduction of systemic blood pressure. The absence of a rise of GFR and the observed increase of fractional sodium excretion indicates that the natriuresis on felodipine is a consequence of a decrease of tubular sodium reabsorption. As others[15], we cannot readily explain the natriuretic effect of felodipine from changes in RPF, FF or RVR, since we found no correlations between changes in these parameters of renal hemodynamics and the increase of sodium excretion on felodipine. As will be discussed in more detail elsewhere in this issue, the mechanism of the natriuretic effect of felodipine still remains unclear. Whatever the mechanism may be, it might contribute to the chronic antihypertensive effect of calcium entry blockers as suggested before, since sodium balance remains negative during long term administration of dihydropyridine calcium entry blockers[4,7]. In the hypertensive patients with renal disease the role of the natriuretic capacity of felodipine in its antihypertensive effect may be more prominent than suggested by the relatively small increase of sodium excretion in this study, since at this level of blood pressure reduction through peripheral vasodilation sodium retention was to be expected[2,3]. Such sodium retention, observed during the use of directly acting vasodilators other than calcium entry blockers, is supposed to antagonize the antihypertensive effect of these drugs[2,3]. Finally, the natriuretic effect of felodipine might reduce the incidence of edema formation, often observed during the administration of vasodilating agents[3,22].

In conclusion, felodipine has an acute natriuretic effect in hypertensive patients with renal disease, albeit possibly less at lower GFR. The very effective blood pressure reduction by felodipine probably attenuated the natriuretic effect of this drug in our patients. Conversely, it is not clear to what extent this natriuretic effect contributes to blood pressure lowering by felodipine. The felodipine-induced increase in sodium excretion is accompanied by decreases of FF and RVR and, in healthy volunteers, by an increase of RPF. However, the natriuretic effect cannot readily be explained by these changes in renal hemodynamics and needs further elucidation.

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CHAPTER 7

Influence of salt intake on the hypotensive and natriuretic responses to felodipine

Henk W. van Hamersvelt, Jack F.M. Wetzels, Heinrich J. Kloke, Robert A.P. Koene, and Frans Th.M. Huysmans.

Submitted

ABSTRACT

Calcium entry blockers (CEB) such as felodipine are powerful arteriolar vasodilators which also induce natriuresis. Several studies have suggested that CEB reduce blood pressure more effectively at higher salt intakes, which might be due to a larger natriuretic effect.

We therefore studied the hypotensive and natriuretic responses to felodipine during low salt (LS, 50 mmol NaCl/day) and high salt diet (HS, 300 mmol NaCl/day) in 12 hypertensive outpatients. Placebo was given the first week of randomly allocated diet periods. The acute effects of felodipine oral solution 5 mg were studied on the eighth day. During the second week of each diet, felodipine 5 mg bid was given to study its chronic effect.

At the end of the placebo period, mean arterial pressure (MAP, Dinamap) was higher during HS than during LS (120 ± 4 and 115 ± 2 mm Hg, $P<0.02$). Felodipine oral solution induced comparable hypotensive responses during HS (-12 ± 1 %) and LS (-11 ± 1 %) despite a greater sodium loss during HS ($+288\pm54$ μmol/min above baseline compared to $+108\pm15$ during LS, $P<0.01$). Also chronic felodipine decreased MAP to the same degree during HS (-10 ± 2 %) and LS (-9 ± 1 %). The lowest MAP was thus reached when using felodipine during LS (103 ± 2 vs. 109 ± 3 mm Hg at HS, $P<0.02$). During both diets, chronic felodipine decreased body weight (-0.5 ± 0.2 kg) and increased foot volume ($+1.7\pm0.8$ %). The highest foot volumes and visible edema were observed with felodipine at HS. Felodipine did not change plasma volume, ANP, or serum albumin, but the diet-induced differences in these parameters had disappeared after felodipine.

In conclusion, felodipine induced comparable hypotensive responses during different salt intakes despite a larger natriuretic effect at HS. The combination of LS and felodipine resulted in the lowest blood pressure and the least edema. We therefore would not advise unrestricted salt intake during treatment with CEB.

INTRODUCTION

Calcium entry blockers (CEB), especially dihydropyridines such as felodipine, are effective antihypertensive drugs with few side effects[1]. In contrast to the sodium retaining effect of other vasodilators[2], CEB have a well established acute natriuretic effect in both normotensive volunteers and hypertensive patients[3]. Although it has been questioned whether this acute sodium loss is maintained in the long term, most sodium balance studies performed after initiation[4-6] or discontinuation of a CEB[7-9] favour a long term reduction in sodium balance. It is still unclear, however, whether the natriuretic effect contributes to the hypotensive response to CEB. It is also a matter of discussion whether dietary sodium intake influences the antihypertensive effect of these drugs[10,11]. Several[5,12,13], but not all[14] studies have indicated that the hypotensive response to CEB is larger during a salt supplemented diet than during dietary salt restriction. Theoretically, such an enhanced hypotensive response during high salt intake might be explained by a larger natriuretic effect[12] or, alternatively, to an enhanced reactivity of vascular smooth muscle cells[15]. In the present study in hypertensive patients, we evaluated the influence of dietary salt intake on the acute and chronic hypotensive and natriuretic responses to felodipine. Several indices of intravascular volume were measured to estimate the long term changes in sodium balance.

METHODS

Patients

For this study, we selected patients with established hypertension and a diastolic blood pressure above 95 mm Hg without antihypertensive treatment. Patients with signs of secondary hypertension, ischemic heart disease, cerebrovascular disease, diabetes mellitus or renal failure (endogenous creatinine clearance below 80 ml/min/1.73 m^2) were excluded. One female with essential hypertension and coexisting benign glomerular haematuria was included. The study protocol was approved by the hospital Ethics Committee.

Twelve white patients (three women) who gave written informed consent entered the study at least two weeks after withdrawal of all antihypertensive drugs. On entry, blood pressure was 167/103 (SD 19/5) mm Hg by mercury

sphygmomanometry. Mean age was 50 years (range 36-65) and mean body mass index 26 kg/m^2 (range 21-32). Nine patients were free of medication, whereas two patients continued the use of diazepam and allopurinol, respectively. Another patient using low dose phentolamine for urologic problems was later excluded from data analysis because of non-compliance with the sodium restricted diet (see results). Three patients occasionally used acetaminophen for headache.

Study protocol

In random sequence, a low salt (LS, 50 mmol NaCl/day) and a high salt diet (HS, 300 mmol NaCl/day) was prescribed for periods of two weeks, separated by an interval of at least four weeks. LS intake was reached by dietary advise and HS intake by adding on average 15 Slow Sodium tablets daily (Ciba Geigy, Basel, CH, 10 mmol NaCl/tablet) to a sodium constant diet. Adherence to the diets was tested by measuring creatinine, sodium and potassium concentrations in 24-hour urine collections. Urine sodium and potassium excretions were adjusted to individual mean 24-hour creatinine excretions.

In a single blind manner, placebo was given the first week and felodipine the second week of each diet period. Felodipine extended release 5 mg (Plendil^R) was given twice daily in order to obtain stable plasma drug levels throughout 24 hours[16]. The influence of the diet *per se* and the acute effects of felodipine oral solution 5 mg were determined on the eighth day, whereas the chronic effects of felodipine were measured on the fifteenth day of both diets. On the eve of all measurements, 600 mg of lithium carbonate (16.2 mmol Li^{2+}) was given orally. Patients were asked to refrain from smoking and the use of alcohol for the last 24 hours and from caffeine-containing beverages for the last eight hours preceding measurements.

On the eighth and the fifteenth day, patients consumed a light breakfast, drank 250 ml of tap water and took their last placebo or felodipine tablet one hour before arrival at the ward, where measurements were performed always in the same room and at the same time of the day. Upon arrival, foot volume was recorded with a recently developed, accurate method using a sensitive balance to measure the water displacement induced by immersion of the foot (intra-individual coefficient of variation 1.31 % for day to day variability). Body weight was measured after voiding and diuresis was induced by a fixed water intake of 375 ml in 60 min. A baseline blood sample was collected and supine

blood pressures and pulse rates were recorded at two min intervals for 30 min with an automatic device (Dinamap model 1846P, Critikon, Tampa, Florida, USA). The mean values of the last 10 recordings were used for analysis. Immediately after the supine rest, another blood sample was taken for determination of hormones. Subsequently, plasma volume was measured by standard isotope dilution technique[17]: 2 μCi of radioiodinated human serum albumen (^{125}I-HSA) was injected intravenously. Before injection and four times at five min intervals thereafter, blood samples were collected from the contralateral arm for determination of plasma ^{125}I concentrations. Another blood and urine sample was collected for estimation of renal clearances.

Only on the eighth day, the experiment continued with the gift of felodipine 5 mg oral solution and an additional oral water load of 450 ml in 60 min. Supine blood pressures and heart rates were recorded at two min intervals 0-60 and 120-150 min after felodipine. At the end of these supine recordings, blood and urine samples were collected for measurement of renal clearances. Foot volume, body weight and hormones were measured 150 min after felodipine oral solution.

In blood and urine samples, creatinine, sodium, potassium, chloride, urate, and phosphate concentrations were measured by standard (semi)automated techniques and lithium by atomic absorption spectrophotometry. Hemoglobin and hematocrit (Ht) were determined by routine Coulter Counter, serum albumin by an automated bromine cresol green method, and plasma renin activity (PRA) with the Phadebas angiotensin I test (Pharmacia Diagnostics, Sweden). Atrial natriuretic peptide (ANP) and plasma aldosterone concentration (PAC) were measured by radioimmunoassays[18,19] and plasma felodipine levels by gas chromatography[20].

Urinary excretions (U_xV), clearances (Cl_x), and fractional excretions (FE_x) were calculated according to standard formulas[21], using Cl_{creat} as an estimate of glomerular filtration rate. Fractional proximal sodium reabsorption (FPR_{Na}) and fractional distal sodium reabsorption (FDR_{Na}) were calculated by the lithium clearance method[22]:

$$FPR_{Na} = (1\text{-}FE_{Li})x100\ (\%).$$
$$FDR_{Na} = (1\text{-}Cl_{Na}/Cl_{Li})x100\ (\%).$$

Statistical analysis

Statistics were performed with SAS (Statistical Analysis System) software. The possibility that the order of the diets influenced various parameters was tested by standard two-sample T-tests[23]. Two tailed Wilcoxon's rank sum test was used for all pairwise comparisons. Probability values below 0.05 were considered statistically significant. Results are presented as means±SEM and "NS" indicates *P* values above 0.10.

RESULTS

Side effects and compliance

The most common side effect of felodipine oral solution was headache (10 patients during either diet), whereas dizziness (2) and flushing (1) were noted in the HS period. The only side effect that was reported more frequently with chronic felodipine than with placebo was edema in the HS (3), but not in the LS period.

All participants completed the study protocol. However, one male subject was excluded from data analysis because of noncompliance with the sodium restricted diet (average urine Na^+ 252 mmol/day). The 11 remaining patients all had a urinary sodium excretion below 100 mmol/day with LS and above 200

Table 1. *Steady state 24 hour urinary excretions during placebo and during felodipine.*

		Low salt	High salt	*P* value*
Urinary volume *(ml/24 h)*	Plac	1634±136	1833±116	NS
	Felo	1737±182	2043±100	NS
Urinary Na^+ *(mmol/24 h)*	Plac	51±7	279±22	<0.01
	Felo	69±8	301±20	<0.01
Urinary K^+ *(mmol/24 h)*	Plac	78±5	75±5	NS
	Felo	81±6	80±5	NS

Values (means±SEM) are averages for the last two days of placebo (plac) and the last two days of felodipine (felo).

*P values for differences between low and high salt diet.

None of the differences between felodipine and placebo reached significance ($P>0.10$).

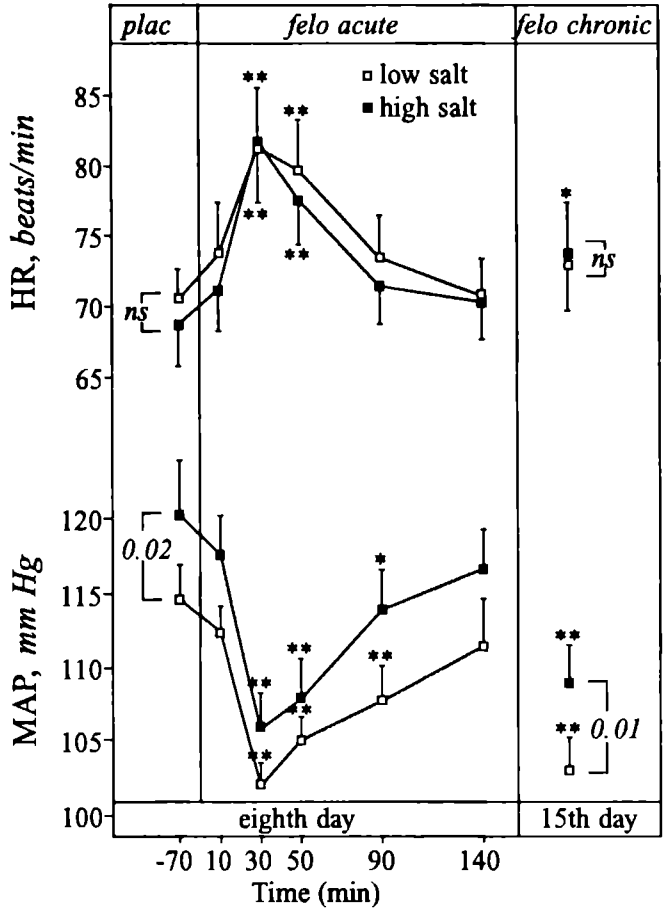


Figure 1. Course of mean arterial pressure (MAP) and heart rate (HR) on two different diets. Baseline values (-70 min) were measured after one week of placebo (plac, eighth day). Acute effect of felodipine (felo) oral solution 5 mg (eighth day) and chronic effect of one week felodipine 5 mg bid (15th day) are illustrated. NS ($P > 0.10$) or numbers regard differences between low and high salt diets. $^{*}P < 0.05$ and $^{**}P < 0.01$ compared to baseline values. Error bars indicate SEM.

mmol/day with HS. Compliance to both diets was as good during felodipine as during placebo (Table 1). Daily urinary volumes and urinary potassium excretions did not differ between LS and HS (Table 1).

Table 2. *Effect of placebo and chronic felodipine on body weights, foot volumes and indices of intravascular volume.*

		Low salt	High salt	*P* value*
Body weight *(kg)*	Plac	76.6±2.6	77.9±2.7	0.02
	Felo	76.1±2.5†	77.4±2.7†	<0.01
Foot volume *(ml)*	Plac	1213±44	1237±43	<0.01
	Felo	1232±40	1258±43†	<0.01
Plasma volume *(ml)*	Plac	2800±111	3034±133	<0.01
	Felo	2928±129	2977±142	NS
Serum albumin *(g/l)*	Plac	45.8±0.6	44.5±0.8	0.03
	Felo	44.7±0.7	45.1±0.6	NS
Hematocrit (%)	Plac	43.3±1.0	41.6±0.9	0.08
	Felo	41.8±0.8	41.4±0.8	NS

Values are expressed as means±SEM. Plac=placebo; Felo=felodipine.
*P values for differences between low and high salt diet.
†$P<0.05$ for the difference between placebo and felodipine.

Effects of salt intake

The order of the two diets appeared to change their effect on hemoglobin and Cl_{creat} (both $P<0.05$ for period effect), implicating that the influence of the diet on these parameters could not be evaluated.

On the eighth day after one week of placebo, supine mean arterial pressure (MAP) was higher during HS than during LS (120±4 and 115±2 mm Hg, respectively, $P<0.02$, Fig. 1), whereas heart rates did not differ. In four patients, the MAP was at least 5 (range 7-25) mm Hg higher during HS than during LS. Body weight, plasma volume, and also foot volume were higher during HS, whereas serum albumin was lower in this period (Table 2). As expected, PRA and PAC were lower and ANP was higher during HS than during LS (PRA: 0.63±0.16 vs. 1.47±0.28 nmol/l/h; PAC: 0.19±0.02 vs. 0.56±0.11 nmol/l; ANP: 12.1±1.7 vs. 8.0±1.0 pmol/l, all differences $P<0.01$, Fig. 2).

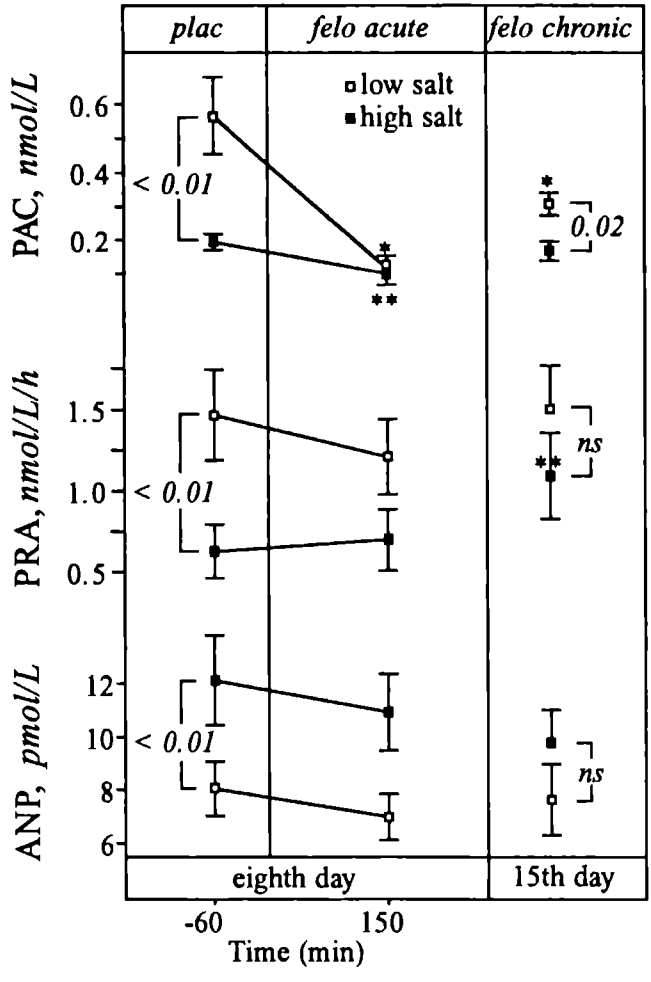
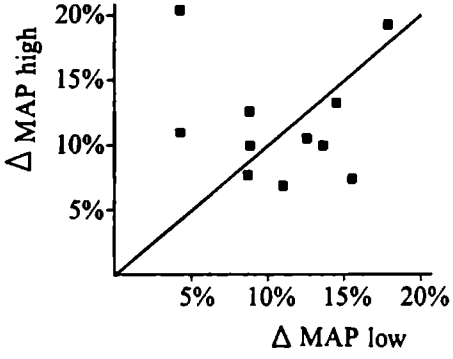


Figure 2. Acute and chronic effects of felodipine on plasma aldosterone concentrations (PAC), plasma renin activity (PRA), and atrial natriuretic peptide (ANP) on two different diets. For further details see Figure 1.

Acute effects of felodipine

Felodipine oral solution induced a rapid fall of MAP accompanied by a rise of heart rate during both diets (Fig. 1). The maximal antihypertensive effect was observed 30 min after felodipine administration and was comparable during the

a) Felodipine acute



b) Felodipine chronic

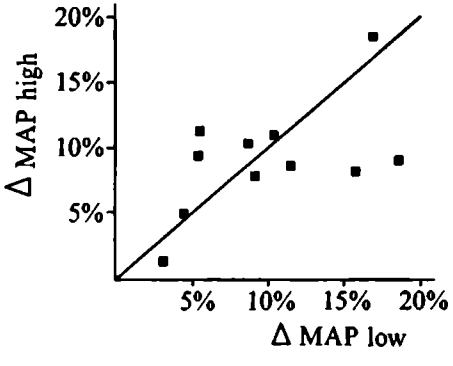


Figure 3. Comparison of individual changes of mean arterial pressure during low (Δ MAP low) and high salt (Δ MAP high) diets after acute (a) and chronic (b) felodipine administration. Oblique lines indicate line of identity.

two diets (HS -12±1 % and LS -11±1 %, Fig. 3). Also, the maximal increases of heart rate did not differ (HS +20±6 % and LS +15±4 %).

Felodipine induced similar and short lasting increases of Cl_{creat} during HS and LS (+27±8 % and +22±8 %) in the period that acute increases of urinary flow rate were observed. This diuretic effect was accompanied by acute increases of Cl_{Na} and FE_{Na} during both diets (Fig. 4). Felodipine induced a greater sodium loss during HS (+288±54 μmol/min above baseline compared to +108±15 μmol/min during LS, $P<0.01$). The relative increase of FE_{Na}, however, was larger during LS (+169±48 % vs. +89±20 % with HS, $P<0.05$). The changes of FE_{Cl} parallelled the changes of FE_{Na} (data not shown). Felodipine induced no significant changes of potassium excretion during either diet (Fig. 4). Pretreatment urate, phosphate, and lithium clearances fitted a more pronounced proximal as well as distal sodium reabsorption during LS (Table 3). During the two diets, felodipine induced comparable increases of FE_{PO4} and decreases of FDR_{Na}. The felodipine-induced increase of FE_{urate}, however, was blunted during LS and also the decrease of FPR_{Na} tended to be less during LS than during HS (Table 3).

In accordance with a larger total urinary volume loss during HS (1158±106 ml vs. 881±46 ml during LS, $P<0.01$), a larger decrease of body weight was observed after felodipine in this period (-1.0±0.1 kg vs. -0.7±0.0 during LS, $P<0.01$). Felodipine did not change foot volume during the period of supine rest in the experiment. There were no significant changes of Ht or

serum albumin with felodipine. ANP and PRA also did not change in contrast to the fall of PAC (Fig. 2).

Plasma felodipine levels were comparable during LS and HS (Fig. 5).

Chronic effects of felodipine

One week of oral felodipine resulted in the same blood pressure decrease during LS and HS (-9±1 % compared to -10±2 %, P=NS, Fig. 3). The diet-induced difference in blood pressure persisted during felodipine and the lowest level of MAP was thus reached with felodipine at LS (103±2 compared to 109±3 mm Hg during felodipine at HS, $P<0.02$, Fig. 1). Heart rate was slightly higher on felodipine than on placebo, but the difference was only significant during HS (Fig. 1).

Felodipine decreased body weight by 0.5±0.2 kg in both diet periods and at the same time increased foot volume, although during LS this increase failed to reach significance (+19±9 ml, P=0.08, Table 2). During LS, but not during HS, felodipine tended to increase plasma volume (+128±55 ml, P=0.07) and decrease serum albumin (-1.1±0.5 g/l, P=0.08) and hematocrit (-1.6±0.6 %, P=0.05). Thus, the diet-induced differences in plasma volume, serum albumin,

Table 3. *Acute effects of felodipine oral solution on several parameters of segmental tubular sodium reabsorption.*

		Low salt	High salt	P value*
FE_{urate} (%)	Baseline	7.1±0.7	8.8±1.2	0.02
	Felo	+0.7±0.5	+2.1±0.6†	0.01
FE_{PO4} (%)	Baseline	8.8±1.7	10.8±2.1	0.04
	Felo	+5.3±0.9‡	+6.0±0.9‡	NS
FPR_{Na}§ (%)	Baseline	81.2±1.1	74.7±2.7	0.01
	Felo	-3.0±0.8‡	-5.2±1.0‡	0.07
FDR_{Na}§ (%)	Baseline	97.8±0.3	92.5±1.0	<0.01
	Felo	-2.8±0.5‡	-3.9±0.8‡	NS

Values are expressed as means±SEM. Baseline=values measured on the eighth day at the end of placebo and just before felodipine administration; Felo=mean change in the first 2½ hours after felodipine oral solution.

*P values for differences between low and high salt diet.

†$P<0.05$ and ‡$P<0.01$ for the acute effect of felodipine oral solution.

§ FPR_{Na} and FDR_{Na} concern nine patients.

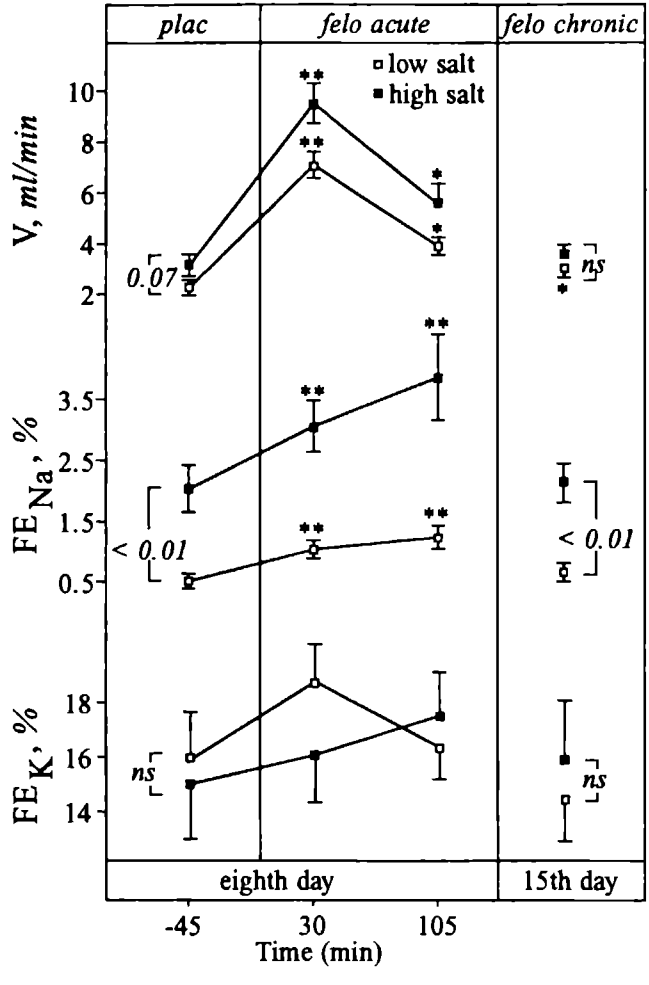


Figure 4. Acute and chronic effects of felodipine on urinary flow rate (V), fractional sodium excretion (FE_{Na}), and fractional potassium excretion (FE_K) on two different diets. For further details see Figure 1.

and hematocrit were no longer demonstrable after felodipine (Table 2). Also the diet-induced differences in ANP and PRA had disappeared (Fig. 2). Felodipine increased PRA during HS and decreased PAC during LS.

Plasma felodipine levels were within the therapeutic range (4-14 nmol/l) and comparable during LS and HS (Fig. 5).

DISCUSSION

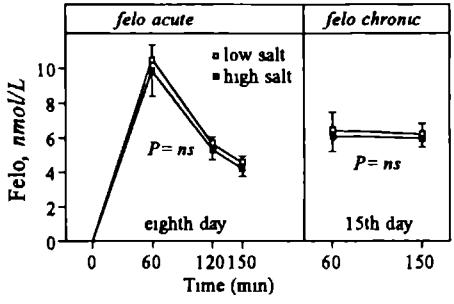


Figure 5. Plasma felodipine levels after acute and chronic felodipine administration on two different diets. P-value on the 8th day for area under the curve on the 15th day for mean values. For further details see Figure 1.

It has been claimed that CEB, unlike most other antihypertensive drugs, reduce blood pressure more effectively at high than at low salt intake[5,12,13]. Such an effect might be ascribed to a larger natriuretic effect of CEB in the salt loaded state. We therefore studied the hypotensive and natriuretic effects of the dihydropyridine CEB felodipine during different dietary salt intakes. In our outpatients with essential hypertension, felodipine oral solution induced comparable acute hypotensive responses during high and low salt intakes. Also one week of continued felodipine treatment resulted in similar reductions of blood pressure in both dietary periods. Since the different salt intakes had resulted in a moderate difference in blood pressure before the administration of felodipine, the lowest blood pressure was achieved using felodipine during dietary salt restriction. Therefore, at first sight, our data seem to contrast with previous claims of a larger antihypertensive effect at high salt intake[5,12,13]. However, these claims were based on the acute hypotensive responses to the dihydropyridine nifedipine, whereas the studies addressing the more chronic effect of CEB during different salt intakes are more in line with our observations. In hospitalized patients, several CEB induced a comparable chronic antihypertensive effect at extremes of salt intake[24,25], whereas only nifedipine caused a slightly, but not significantly smaller hypotensive response during severe sodium restriction[5]. Moreover, in one of these studies, the lowest blood pressure was reported with the dihydropyridine nisoldipine at low salt intake[25]. In contrast, Luft et al could not demonstrate an additional hypotensive effect of moderate sodium restriction in patients already treated with nifedipine[9]. However, in this study, sodium restriction was applied for six days only, whereas we have prescribed diets for two weeks. Possibly, such a longer period of moderate sodium restriction is needed to achieve maximal effect on blood pressure. Because of the fixed order of placebo and felodipine in our study, we cannot exclude that the dietary salt intake had additional effects on blood pressure during the second week of each diet period

when felodipine was used. Part of the observed difference in final blood pressure during chronic felodipine might thus be ascribed to a late effect of the diets, but this does not weaken our conclusion that the most favourable blood pressure is reached with felodipine and moderate dietary salt restriction.

The discrepancies between our study and the aforementioned acute studies with nifedipine[5,12,13] might be explained by differences in study protocols, patient populations, or dose and type of drug used. In only four of our patients, mean arterial pressure was more than five mm of mercury higher during high salt than during low salt intake. In contrast, in the studies with nifedipine, a rigorous sodium depletion[5,12] and/or inclusion of more so called sodium sensitive patients[12,13] had resulted in more pronounced differences in blood pressure between high and low salt intake. Under such circumstances, differences in antihypertensive response are to be expected since the fall in blood pressure with any antihypertensive agent depends directly on the pretreatment level[26,27]. In this respect it is rather surprising that a relatively high dose of the dihydropyridine CEB nicardipine induced similar acute hypotensive responses at extremes of salt intake in markedly sodium sensitive Japanese patients[14].

We clearly demonstrated that felodipine acutely increases diuresis and natriuresis despite the simultaneous reduction of blood pressure, thereby confirming previous observations with other CEB[3]. It has been suggested that this natriuresis might contribute to the blood pressure lowering action of CEB[28], but our data do not substantiate this idea. First, during high salt intake felodipine induced a larger sodium and weight loss than during low salt intake, but this was not reflected in a larger reduction of blood pressure in the high salt period. Also the time course of blood pressure and sodium excretion (Fig. 1 and 4) argue against a major role for the natriuretic effect in the acute hypotensive response to CEB. Furthermore, it has been shown that nifedipine reduces blood pressure during severe, diuretic-induced sodium depletion, a condition where nifedipine no longer exerts a natriuretic effect[12].

Our data on segmental tubular sodium reabsorption indicate that dietary salt restriction enhances proximal and distal sodium reabsorption. Under these circumstances felodipine had a smaller natriuretic effect than on the high salt diet, which appeared to be mainly due to less inhibition of proximal reabsorption on the low salt diet. Somewhat in contrast, felodipine had comparable effects on distal tubular sodium reabsorption and urinary potassium excretion, which is surprising in view of the differences in plasma aldosterone levels before administration of felodipine oral solution. However, plasma aldosterone levels were no longer different after felodipine due to a pronounced fall of aldosterone

in especially the low salt period. Also the smaller distal tubular sodium load after felodipine in the low salt period might have been responsible for the absence of a difference in distal tubular effects of felodipine between the two diets.

After one week of continued treatment with felodipine, body weight was 0.5 kg lower than after placebo in both diet periods. This decrease of body weight corresponds with a sodium loss of approximately 75 mmol, which is in agreement with the data from sodium balance studies during calcium entry blockade[6-8]. These data imply that treatment of hypertension with CEB is clearly not associated with sodium retention, a well known side effect of other arteriolar vasodilators. Both in the acute and the chronic phase, the reduction of body weight by felodipine was not reflected in significant changes of plasma volume or the other indirect indices of intravascular volume. From this we must conclude that fluid and sodium losses are mainly derived from the extravascular compartment. Interestingly, the diet-induced differences in plasma volume, serum albumin, hematocrit, ANP, and PRA had disappeared after continued felodipine administration, although the difference in body weight was maintained. This effect of felodipine appeared to be due to a slight rise in plasma volume during the low salt diet rather than a decrease in plasma volume during the high salt diet. This suggests that at different salt intakes CEB have different effects on the distribution of fluid between the intra- and extravascular compartment. Although there is no ready explanation for such an effect of CEB, we would suggest that the lower blood pressure level with felodipine at low salt intake favours a shift of fluid into the intravascular compartment which apparently does not occur at the somewhat higher blood pressure level observed with felodipine at high salt intake.

Felodipine induced a comparable degree of edema formation during high and low salt intake, but the additive effects of felodipine and high salt diet resulted in the largest foot volume. Accordingly, clinical ankle edema with felodipine was noted in three patients during the high salt period. The edema formation with felodipine was not accompanied by any other sign of sodium retention and occurred despite a simultaneous reduction in body weight. These data support the hypothesis that edema formation with CEB is a local phenomenon at the site of vasodilation: preferential dilation of small, precapillary arterioles[29,30] presumably leads to an increase in intracapillary hydrostatic pressure which explains the observed increase of transcapillary fluid filtration in skeletal muscles[31,32]. In the present study, felodipine did not induce acute changes of foot volume in the supine patients in contrast to our observations with nifedipine in

healthy sedentary volunteers[33]. This discrepancy may be explained by interference of nifedipine with the increase in vascular resistance which normally protects capillaries against an orthostatic load[31]. The edema formation in the depending legs also implies a larger decrease of extravascular volume in the other parts of the body than already assumed in the previous paragraph. Because dihydropyridines preferentially increase skeletal muscle, coronary, and cerebral blood flow[34], a decrease of extravascular volume in the other organs might be assumed.

Although we did not observe renal sodium retention after one week of felodipine, it is possible that long-term administration would have led to ongoing edema formation followed by renal sodium retention. Such a mechanism could explain the observation of an increase of exchangeable sodium without a change of plasma volume after eight weeks of nifedipine[35]. With a high dose of a thiazide diuretic, leg edema disappeared and exchangeable sodium normalized at the cost of a considerable reduction in plasma volume[35]. Our results show that the incidence of edema can also be reduced by restricting dietary salt intake. Both measures are probably less effective than in other edematous states because the edema with CEB is not primarily due to sodium retention.

In conclusion, felodipine induced comparable acute and chronic hypotensive responses during different salt intakes despite a somewhat larger natriuretic effect with the high salt diet. The combination of modest sodium restriction and felodipine resulted not only in the lowest blood pressure but also in less edema. We therefore would not advise unrestricted salt intake for patients on CEB.

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CHAPTER 8

A new method for measuring human foot volume: accuracy and biological variation

Henk W. van Hamersvelt, Dirk J. de Jong, Heinrich J. Kloke,

Ignace M. Valk, Robert A.P. Koene,

and Frans Th.M. Huysmans.

Submitted

ABSTRACT

In pathological conditions mainly characterized by ankle edema, regular and reliable estimates of foot volume are needed for follow-up, especially after therapeutic interventions. Most available devices are inaccurate or not suited for long-term studies.

We describe a new method that uses a sensitive balance to measure the force needed for immersion of the foot in a water-bath. The degree of filling of this water-bath is controlled with the same balance, which enables refreshing of the water with only minimal errors (coefficient of variation (CV)=0.09 % for standard measure). Accuracy of the method was demonstrated by repeated measurements of foot volume in volunteers (CV=0.32 %, n=27) and in patients with various renal diseases (CV=0.28 %, n=22). Foot volume remained stable during at least 10 minutes in water of room temperature, which makes a heating device unnecessary. Biological changes were demonstrated easily: foot volume was increased by 15 minutes sedentary rest (+1.0±0.2 %, n=9), and in the course of the day (+1.2±0.5 %, n=12). It was also higher premenstrually (+1.2±0.5 %, n=7). Day to day variation, measured at the same points of time, was considerable (CV=1.31 %) and should be taken into account in long-term studies.

INTRODUCTION.

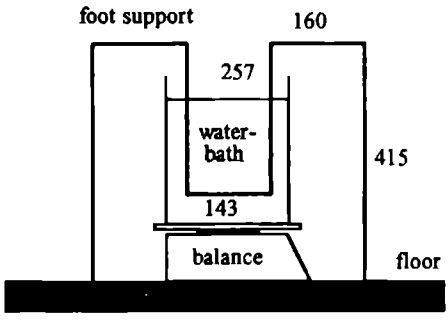
Ankle edema is a common symptom in several pathological states such as chronic venous insufficiency, congestive heart failure, and the nephrotic syndrome. In conditions where the amount of edema is the only available parameter for evaluation of the course of the disease and the effect of therapeutic interventions, it is important to have an accurate and simple method to quantitate the volume of the foot. Most available methods estimate foot volume by recording water displacement after immersion of the foot. Some methods measure water overflow[1-3], whereas others determine the rise of the water-level on a calibrated scale[4,5] or with more sophisticated devices[6,7]. Yet another method uses the principle of Archimedes, i.e. upward force equals the weight of the displaced water, and determines the water pressure at a fixed point in the foot-bath[8]. All these methods have important disadvantages: some lack accuracy whereas the others are not suited for measurements over a prolonged period of time. Here, we describe a new, accurate, sensitive, and simple method which also uses the principle of Archimedes and is suited for repeated measurements of foot volume. We have tested the accuracy of this method and have evaluated the influence of biological variables on measured foot volumes.

METHODS

Apparatus and procedure

Similar to the other methods for measuring foot volume, our new method (I.M. Valk) uses the principle of water displacement, but records it in a new, indirect and precise way. As shown in Figure 1, a perspex water-bath with a maximal capacity of 20 l is placed on a sensitive electronic balance (Mettler PM 34, Mettler Instrumente AG, Greifensee, Switzerland). A specially constructed stainless steel foot support is suspended in the foot-bath, but rests fully on the floor on both sides of the balance. The water-bath is filled with tap water until the balance indicates exactly 15,000 g, thereafter the balance is tared to zero. Subsequent immersion of the foot induces water displacement that is perceived by the balance as an increase in weight, which equals the immersed volume multiplied by the relative weight of water at a specific temperature (0.998 g/ml at 22 °C). Stated differently, the balance perceives the force that is necessary

a) Foot volume recorder



b) Bottom of foot support (immersed part)

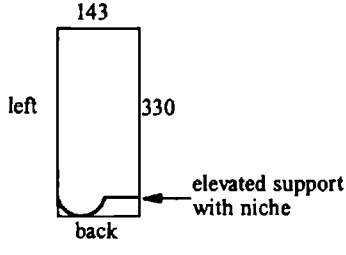


Figure 1. Side view (a) and top view (b) of foot volume recorder. Measures of foot support (in mm) are indicated. Perspex water-bath measuring 388x208x250 mm had a maximal capacity of 20 litres. Left foot of the subject is placed on the bottom of the foot support with the heel against the niche in the back.

to immerse the foot and which depends solely on the volume of the foot (Archimedes' principle). In order to reach a fixed and reproducible degree of immersion of the foot and lower leg, a stable chair with adjustable height is used and a rigidly standardized seated position is pursued with the thigh horizontal, lower leg vertical, and left foot resting on the foot support with the heel against the niche in the back and the fore-foot against the left side of the foot support (see Figure 1b). Moreover, to minimize errors due to different degrees of immersion, three or four successive foot placements and recordings are made without actually removing the foot from the water-bath: if less than three recordings are within a 5 g range, the foot is withdrawn and the whole procedure is repeated. However, this proved to be seldom necessary and in most persons foot volume can be measured within one minute.

Removal of the foot from the water-bath and evaporation cause water losses between experiments. Such losses are easily corrected for by adding water to the bath until the balance indicates again exactly zero. Thus, the same water-level is reached before each measurement. Unless stated otherwise, water of room temperature was used (20-24 °C). Because the relative volume of water closely approaches 1 at room temperature (1.00223 ml/g at 22 °C), the simple conversion of 1 g to 1 ml can be used since it systematically underestimates volume by only 0.2 %.

Subjects

Fifty-seven healthy volunteers (15 F), aged 19-34 years, participated in one or more of the substudies. All subjects were without signs of venous insufficiency and had a normal length (range, 1.62 to 1.97 m) and body weight (range, 49 to 95 kg). Additionally, 22 patients (6 F) with a mean age of 49 years (range, 17 to 83 years) were selected from our out-patient department to test the reproducibility of the method. Most patients had chronic renal diseases and three of them had large amounts of edema due to either a nephrotic syndrome (2) or fluid overload caused by advanced renal failure (1). Mean length was 1.76 m (range, 1.60 to 1.96 m) and mean body weight was 73 kg (range, 59 to 97 kg).

The study protocol was approved by the Hospital Ethics Committee and informed consent was obtained from all participants.

Reproducibility

Refilling the water-bath. The maximal error induced by refreshing the water in the water-bath was estimated by refilling the water-bath 10 times and subsequently measuring the water displacement of a standard measure (a bottle).

Foot volume. To estimate the error of the method for measuring foot volume, three successive recordings were made within approximately five minutes immediately after arrival at the ward in 27 volunteers (9 F) and in all 22 patients. The foot was withdrawn from the water-bath between recordings and carefully dried. The water lost by removal of the foot was substituted before the next recording as indicated above.

Biological variation

Sedentary rest. Foot volumes were measured in 9 male volunteers immediately after arrival at the ward and after 15 and 30 minutes of sedentary rest.

Water temperature. Changes of foot volume were studied in cold water (14.7 to 16.5 °C), water of room temperature (lukewarm, 20.9 to 23.1 °C), and warm water (33.9 to 37.4 °C) in three groups of 10, 9 and 11 volunteers, respectively. Foot volume was first recorded in water of room temperature in

all subjects immediately after arrival at the ward. During a subsequent sedentary rest of at least 15 min, the water-bath was refilled with water of the desired temperature. Thereafter, subjects immersed their foot for 10 min and foot volume was recorded immediately, and after one, five and ten minutes of immersion. Foot volumes were calculated by multiplying the recorded weight with the relative volume of water at that temperature (1.00090 for cold, 1.00223 for lukewarm, and 1.00600 ml/g for warm water)[9].

Time of the day. Foot volumes were measured in the course of a usual weekday in 12 students (6 F). Recordings were made without a preceding sedentary rest in the morning (range, 8.18 to 10.30 hr), at noon (range, 12.00 to 14.05 hr), and in the afternoon (range, 15.45 to 17.47 hr). Additionally, foot volume was measured after 20 min supine rest following the recording in the afternoon.

Exercise. Immediately after arrival at the ward, 14 volunteers (6 F) performed a 15 min bicycle exercise at 75 % of the estimated maximal capacity (range, 132 to 190 Watt) which was followed by a 15 min sedentary rest. Foot volumes were recorded before and immediately after the exercise and also after the sedentary rest following the exercise.

Menstrual cycle. Foot volumes were determined twice in seven females which had a regular cycle (3) or used oral contraceptives (4). Foot volume was recorded at the same time of the day 0-5 days before and 12-15 days after menstruation.

Day to day variability. Foot volumes were measured in the course of several weeks in 23 volunteers. In 12 of them (2 F), foot volumes were recorded five times in two to five weeks, always at the same time of the day (five in the morning) after a sedentary rest of 30 min. In 11 other male volunteers, foot volumes were measured three times in the course of two to seven weeks without a preceding sedentary rest, but always in the morning within two hours of rising.

Statistical analysis

Statistics were performed with SAS (Statistical Analysis System) software. Coefficients of variation (CV) for reproducibility were calculated by one way analysis of variance[10,11]. The influence of biological variation was evaluated by Wilcoxon's rank sum test for pairwise comparisons and Tukey's studentized range test for multiple comparisons. Probability values below 0.05 were considered statistically significant and results are presented as means $\pm$ SEM.

RESULTS

Reproducibility

Refilling the water-bath. The recorded volume of the standard measure varied only slightly with repeated filling of the water-bath: the CV was 0.09 % at a mean volume of 1111 ml. The actual error induced by refreshing the water will be smaller, because placement of the bottle (the standard measure) on the foot support is also a source of variation in this experiment.

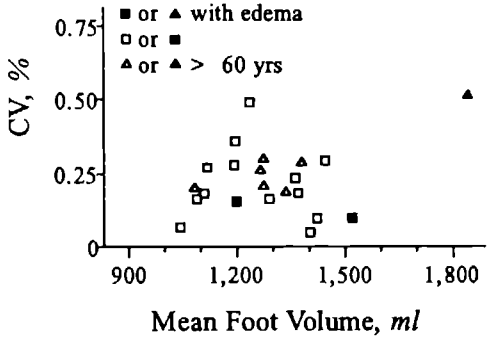


Figure 2. Individual coefficients of variation (CV) for foot volume recordings repeated within a few minutes in 22 patients with various renal diseases. There was no indication that the method was less accurate in older patients or in patients with massive edema.

Foot volume. Our method proved to be accurate with a CV of 0.30 % for measurements repeated within a few minutes. The procedure itself did not influence measured foot volume, because there was no significant difference between the three subsequent recordings. Accuracy of the method was as good in patients as in volunteers with a CV of 0.28 % and 0.32 % at mean foot volumes of 1292 and 1234 ml, respectively. There was no indication that the method was less accurate in patients above 60 years (n=7) or in patients with edema (n=3) (Fig. 2).

Biological variation

Sedentary rest. Resting in the sitting position induced an increase of foot volume from 1305±54 ml on arrival at the ward to 1317±55 ml after 15 min sitting (+1.0±0.2 %, $P<0.01$). Foot volume did not increase further in the subsequent 15 min of sedentary rest (+0.1±0.2 %, $P>0.10$).

Water temperature. In the three temperature groups, comparable increases of foot volume were observed during the 15 minutes sedentary rest preceding the 10 minutes immersion (+0.7±0.3 %, +1.0±0.2 %, and +1.1±0.1 % in the groups assigned to cold, lukewarm and warm water, respectively, differences NS with Tukey's test). During immersion, foot volume remained stable in lukewarm water, whereas it decreased slightly in cold water (-0.3±0.1 % after

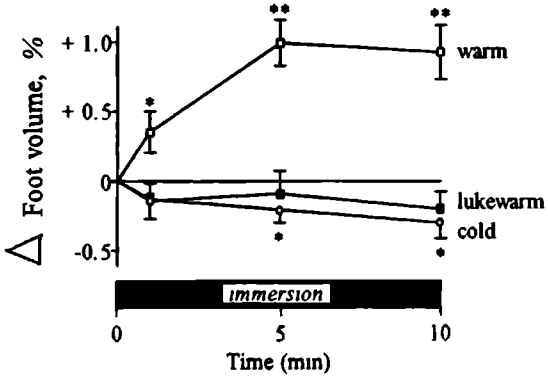


*Figure 3. Relative changes of foot volume during 10 minutes continuous immersion in water of different temperatures. *P<0.05 and **P<0.01 compared to start of immersion. The change in warm water was different from the change in cold water (1, 5 and 10 min), and from the change in lukewarm water (5 and 10 min) (P<0.05 with Tukey's test).*

10 minutes, Fig. 3). The most prominent change, however, was the increase of foot volume in warm water (+0.9±0.2 %, Fig. 3).

Time of the day. Foot volume increased by 1.2±0.5 % in the course of the day and this increase was virtually reversed by the subsequent 20 min supine rest (Fig. 4).

Exercise. The bicycle exercise test itself did not change foot volume (+0.1±0.3 %, $P>0.10$), but the 15 min sedentary rest after the test resulted in a 0.9±0.2 % increase of foot volume ($P<0.01$).

Menstrual cycle. The premenstrual value of foot volume was 1.2±0.5 % higher than the mid-cycle value ($P=0.03$).

Day to day variability. Foot volume varied considerably from day to day (CV=1.31 %, mean volume 1304 ml). The variation appeared to increase in the course of the day, because the subjects measured early in the morning had a lower CV (0.97 %) than the subjects measured either in the morning or in the afternoon (1.42 %), despite the preceding sedentary rest in the latter group.

DISCUSSION

Our new simple method for recording foot volume proved to be accurate with a CV of only 0.30 % for repeated measurements. Most recordings could be made within one minute in both volunteers and patients who were not acquainted with the procedure, without negatively influencing its accuracy.

The reproducibility of our method cannot be easily compared to other methods, either because of insufficient testing of the method[2,3,7] or because of unusual ways to express reproducibility[1,4,6] which do not concur with the recently advised approach[10]. Therefore, we have expressed the reproducibility

in several other ways to make comparisons possible (Table 1). Our method appears to be more accurate than the methods of Thulesius[6] and Christensen[5], which determined the water level with a photo-electric float-sensor and a calibrated scale, respectively. Winkel[4] also used a calibrated scale, but reached a higher accuracy by reducing the free water surface area. However, this method is only accurate for short term changes because total foot volume was measured only once and the differences between successive recordings were determined by the amount of water that had to be added or removed to reach the same mark on the calibrated scale. The error induced by refreshing the water was not tested, but probably could not be controlled easily. In contrast, refreshment of the water in our apparatus will not lead to major experimental errors as indicated by the low

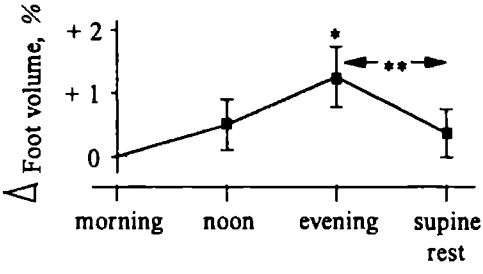


*Figure 4. Relative changes of foot volume in the course of a usual week-day in 12 students, with the last measurement made after 20 min supine rest. *P<0.05 for the change from morning value and **P<0.01 for the change induced by supine rest.*

Table 1. *Comparison of reproducibility of different volumetric methods*

	n	Coefficient of variation[A]			long-term[B]
		Σ|d|/n	√(Σd²/n)	ANOVA	
Winkel[4]	37	0.31 %[C]	-	-	no
Thulesius et al[6]	48	-	1.56 %[D]	-	yes
Christensen et al[5]	9	-	0.70 %[E]	-	yes
van Hamersvelt et al	49	0.31 %	0.38 %	0.30 %	yes

[A] Coefficients of variation were calculated by (SD/mean) x 100 %, with SD calculated according to one of the three methods. √(Σd²/n) and analysis of variance (ANOVA) are the preferred approach with two and more than two repeated measurements, respectively.

[B] suitability of the method for intermittent recordings over prolonged periods of time.

[C] Winkel measured foot volumes at 21 or 31 °C and reported a CV of 0.16 %. However, he omitted the first of three successive recordings at 21 °C because of a supposed "thermal effect". The value in the table has included all recordings.

[D] Thulesius used √(Σd²/2n), so we have multiplied their value of 1.1 % by √2.

[E] Christensen did not report the method used to calculate the CV.

CV of 0.09 % for the volume of the standard measure with repeated filling of the bath. This makes our method also suited for measuring changes over prolonged time intervals. Moreover, by linking the Mettler balance to a personal computer, our method can also be used for continuous recording of foot volume (unpublished observations).

Foot volume remained stable during immersion in water of room temperature, in contrast to the rapid increase in warm water and the slight decrease in colder water. These findings allow future measurements in water of room temperature and thus obviates the necessity of a heating device. The foot swelling in warm water is in agreement with the opinion that a temperature above 34 °C should not be used for volumetry[12]. It has been suggested that the first immersion in water of room temperature reduces foot volume by a "thermal effect" ascribed to decreased pooling of venous blood[4]. However, we did not observe such a decrease during the 10 min immersion and we did not find a difference between the three successive recordings used for testing the reproducibility. We therefore doubt the presence of an acute "thermal effect" (see also Table 1).

Our method was accurate enough to show biological changes that had been expected theoretically. Fifteen minutes sedentary rest was followed by a 1 % increase of foot volume. Foot volume was also higher at the end of a usual week-day in students, comparable to the reported increases of 0.8 % after "active" sitting and 2.3 % after "semi-active" sitting[13]. Such foot swelling in the course of the day underscores the well-known advice to buy your shoes in the afternoon. Foot volume was higher premenstrually which is in accordance with observations on the premenstrual syndrome[14]. In contrast to our expectation, the bicycle exercise did not reduce foot volume, probably because foot volume was already minimal before the exercise, as it was measured immediately after arrival at the ward. A preceding sedentary rest could have provided us the answer whether bicycling reduces foot volume by activation of the calf muscle venous-pump, comparable to the effect of several knee-bends[8].

The procedure that we used proved to be reliable in older patients, who are more likely to suffer from edematous states such as chronic venous insufficiency. Our method is thus suited to study the efficacy of therapeutic interventions for these conditions. Our method might also be useful to follow the short- and long-term effects of hormonal treatment of acromegaly. In such studies, a considerable day to day variability of maximally 5 % (4 x CV of 1.31 %) should be taken into account. Moreover, the day to day variability could be even larger in patients with edema. In one such patient with a nephrotic syndrome we observed an increase of foot volume of 17 % from the

morning of one day to the afternoon of the following day. This example underscores once more the importance to measure foot volume under standardized conditions. Different ambient temperatures could possibly also influence foot volume, but were not studied and cannot be controlled easily.

In conclusion, our new device enables accurate measurements of foot volume and can be used to demonstrate small biological changes. This biological variability should be taken into account if recordings are made over prolonged periods of time. Day to day variability seems acceptable if measurements are made at the same time of the day, preferably early in the morning after a sedentary rest of at least 15 min. Measurements in the premenstrual period should be avoided, unless one is interested in changes during the menstrual cycle.

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CHAPTER 9

Edema formation with the vasodilators nifedipine and diazoxide: local effect or sodium retention ?

Henk W. van Hamersvelt, Heinrich J. Kloke, Dirk J. de Jong,
Robert A.P. Koene, and Frans Th.M. Huysmans.

Submitted

ABSTRACT

Ankle edema is a common side effect of antihypertensive treatment with arteriolar vasodilators such as the calcium entry blocker (CEB) nifedipine and the potassium channel opener (PCO) diazoxide. This edema might be a direct result of peripheral vasodilation or a consequence of renal sodium retention.

We therefore studied during three hours the effects of nifedipine (20 mg), diazoxide (150 mg i.v.), captopril (25 mg), and placebo on edema formation and sodium excretion in 12 healthy sedentary volunteers. The degree of edema was determined by measuring foot volume with a new accurate device. All active drugs decreased diastolic blood pressure (captopril -9±2 %, nifedipine -4±3 %, and diazoxide -2±2 %, all $P<0.05$ compared to +5±2 % with placebo). Foot volume increased acutely after nifedipine (+2.6±0.4 %, $P<0.01$), whereas it remained stable with placebo and the other drugs. Captopril and nifedipine induced increases of fractional sodium excretion (+20±9 % and +40±20 %, $P<0.05$ vs. placebo) in contrast to the decreases with placebo and diazoxide (-13±11 % and -24±10 %). Only nifedipine induced significant albeit small increases of hemoglobin and serum albumin.

In conclusion, nifedipine simultaneously induced acute increases of foot volume and natriuresis, which supports the hypothesis that the development of ankle edema with CEB is a local phenomenon at the site of vasodilation. The absence of a similar increase of foot volume with diazoxide might be explained by less vasodilation in these volunteers but could be indicative for a different mechanism for edema formation with PCO.

INTRODUCTION.

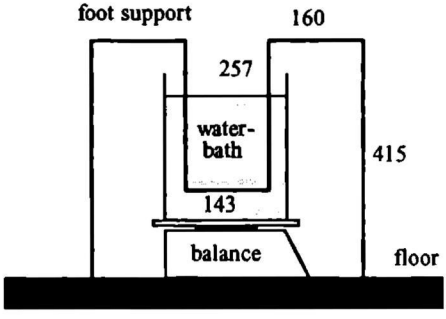
Ankle edema is a common side effect of antihypertensive treatment with arteriolar vasodilators, particularly with calcium entry blockers (CEB) and potassium channel openers (PCO) such as diazoxide[1]. In the heterogeneous group of CEB, the incidence of edema formation is higher with dihydropyridines such as nifedipine than with drugs such as verapamil and diltiazem[2]. Most likely, this difference is related to the higher vascular selectivity and, consequently, the more pronounced vasodilating effect of dihydropyridines[3]. Edema formation with vasodilating drugs might be a direct result of vasodilation, but has also been ascribed primarily to renal sodium retention following blood pressure reduction[4]. This latter possibility seems unlikely for CEB, which do not cause sodium retention[5,6] but even induce an acute natriuresis and diuresis[7]. Moreover, CEB acutely increase transcapillary fluid filtration in skeletal muscles of animals[8] and of humans[9], which may favour edema formation. Dihydropyridine CEB also induce an acute increase of hematocrit in anephric rats which suggests acute extravasation of fluid to the interstitial compartment[10]. All these findings support the hypothesis that ankle edema with CEB and possibly also other arteriolar vasodilators is an immediate consequence of peripheral vasodilation. We have evaluated this hypothesis in humans by studying the acute effects of nifedipine, diazoxide and the ACE inhibitor captopril on foot volume and sodium excretion in healthy sedentary volunteers. Foot volumes were used as an exact measure of the degree of edema formation and were recorded with a recently developed, accurate, sensitive and simple method.

METHODS

Subjects

Twelve healthy volunteers (10 M/2 F), aged 19 to 33 years, were recruited. All participants were free of medication and without signs of venous insufficiency. The two females did not use oral contraceptives. Blood pressures were below 140/90 mm Hg (ranges, systolic 101 to 140 and diastolic 60 to 84 mm Hg) and all individuals had a normal length (range, 167 to 192 cm) and body weight (range, 49 to 95 kg).

a) Foot volume recorder



b) Bottom of foot support (immersed part)

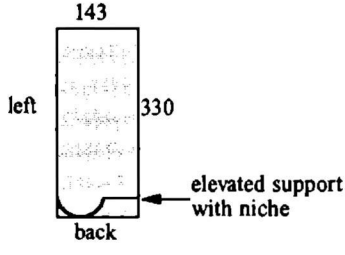


Figure 1. Side view (a) and top view (b) of foot volume recorder. Measures of foot support (in mm) are indicated. Perspex water-bath measuring 388x208x250 mm had a maximal capacity of 20 litres. Left foot of the subject is placed on the bottom of the foot support with the heel against the niche in the back.

The study protocol was approved by the Hospital Ethics Committee and all volunteers gave written informed consent.

Study protocol

The acute effects of the experimental drugs on foot volume and natriuresis were studied on four different days separated by interval periods of at least three days. In a randomized, crossover, open manner all subjects received oral placebo, 20 mg of nifedipine (two capsules of 10 mg bitten and swallowed), 25 mg of captopril (tablet swallowed), and 150 mg of diazoxide (infused i.v. in 10 min). The volunteers continued their usual diet between experiments. Foot volumes were recorded with a recently developed, accurate method with a coefficient of variation of 0.30 % for repeated measurements (manuscript in preparation). In short, a perspex water-bath is placed on a sensitive electronic balance (Mettler PM 34, Mettler Instrumente AG, Greifensee, Switzerland) and a specially constructed stainless steel foot support is suspended in the foot-bath, but rests fully on the floor on both sides of the balance (Fig. 1). Immersion of the foot will induce water displacement and the downward force needed for this water displacement is perceived by the balance as an increase in weight which depends only on the immersed volume. The perceived increase in weight in grams corresponds to the foot volume in ml, since the relative volume of water closely approaches 1 at room temperature (1.00223 ml/g at 22°C). In preliminary studies, we observed an increase of foot volume after the first 15 min of sedentary rest and at the end of the day. To reduce the influence of such

biological variation, subjects were always studied at the same time of the day and baseline levels of foot volume were recorded after 30 minutes of sedentary rest. Only volumes of the left foot were recorded.

Subjects were kept fasting during the last three hours preceding the experiments except for an oral water load of 300 ml taken two hours before drug administration. After arrival at the ward, the volunteers were seated in normal office chairs with their feet resting on the ground. This sedentary rest was interrupted only for foot volume recordings and for voiding in an adjacent room. After 30 min sedentary rest, baseline levels of blood pressure, heart rate, body weight and foot volume were recorded and baseline blood and urine samples were collected. Thereafter, one of the four experimental drugs was administered and subjects drank 250 ml tap water. An additional oral water load of 375 ml was supplied in the following 90 min. Foot volumes, body weights, blood pressures and heart rates were recorded 30, 60, 120 and 180 min after drug administration, whereas blood and urine samples were collected at 60 and 180 min.

Blood pressure and heart rate were recorded in triplicate with a Hawksley random zero mercury sphygmomanometer and by pulse counting, respectively. In blood and urine samples, creatinine, sodium and chloride concentrations were determined by standard (semi)automated techniques. Hemoglobin was determined by routine Coulter Counter and serum albumin by an automated bromine cresol green method. Fractional sodium and chloride excretions (FE_{Na} and FE_{Cl}) were calculated using the formula:

$$FE_x = (U_x/P_x) \times (P_{creat}/U_{creat}) \times 100\ \%$$

with U_x and P_x representing the urinary and plasma concentrations.

Statistical analysis

Statistics were performed with SAS (Statistical Analysis System) software. Repeated measures analysis of variance was used to compare baseline values, whereas Wilcoxon's rank sum test was used to compare the effects of the experimental drugs with placebo. Percentage changes from baseline were used for these pairwise comparisons. Correlation coefficients were calculated according to Spearman. Probability values below 0.05 were considered statistically significant and results are presented as means$\pm$SEM.

RESULTS

Two subjects were unable to empty their bladder at the requested time on one or more occasions. Therefore, comparisons of fractional excretions were restricted to the remaining 10 subjects.

Baseline levels of all measured parameters were comparable on the four experimental days (Table 1).

In these healthy volunteers, captopril decreased systolic blood pressure (-5 ± 2 % vs. 0 ± 1 % with placebo, $P<0.05$), whereas neither diazoxide nor nifedipine had an effect on this parameter (-1 ± 1 % and $+1\pm2$ %, respectively). Therefore, only diastolic blood pressures, which decreased with all three active drugs, are shown in Figure 2. The blood pressure lowering effects of captopril and nifedipine were more pronounced than that of diazoxide, whereas the effect of captopril lasted longest. Neither diazoxide nor captopril had an effect on heart rate (-2 ± 2 % and $+1\pm2$ %), whereas nifedipine increased it by 7 ± 3 % (-1 ± 2 % with placebo, $P<0.05$).

Foot volume remained stable on placebo days and was not changed by either captopril or diazoxide (Fig. 2 and 3). In contrast, foot volume rapidly increased with nifedipine and remained elevated until the end of the experiment three hours after drug administration (mean increase 2.6 ± 0.4 %, $P<0.01$, Fig. 2 and 3). None of the subjects developed visible edema in this period.

Table 1. *Baseline values before drug administration*

	Plac	Capt	Diaz	Nife	P*
Body weight *(kg)*	75.3 ± 3.8	75.4 ± 3.9	75.2 ± 3.8	75.1 ± 3.8	0.09
Systolic BP *(mm Hg)*	106 ± 3	108 ± 2	104 ± 2	105 ± 2	0.46
Diastolic BP *(mm Hg)*	68 ± 2	72 ± 2	68 ± 1	70 ± 2	0.13
Heart rate *(beats/min)*	66 ± 2	65 ± 2	67 ± 2	68 ± 2	0.27
Foot volume *(ml)*	1344 ± 44	1335 ± 42	1335 ± 43	1333 ± 42	0.56
Hemoglobin *(mmol/L)*	8.4 ± 0.1	8.5 ± 0.2	8.5 ± 0.2	8.5 ± 0.2	0.59
Albumin *(g/L)*	48 ± 1	50 ± 1	49 ± 1	50 ± 1	0.24
FE_{Na} *(%)*	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.83
FE_{Cl} *(%)*	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	0.50

Values are means$\pm$SEM. Plac=placebo; Capt=captopril; Diaz=diazoxide; Nife=nifedipine; BP=blood pressure.

*P-values for comparison of baseline values by repeated measures analysis of variance.

Fractional sodium excretion gradually decreased on placebo days and a similar decrease was observed with diazoxide (Fig. 3). In contrast, both captopril and nifedipine induced an increase of natriuresis. Changes of FE_{Cl} parallelled the changes of FE_{Na} with decreases after placebo and diazoxide (-20±12 % and -13±13 %) and increases after captopril and nifedipine (+9±19 % and +22±13 %). The three active drugs did not change serum sodium or urine volume in the three hours after drug administration (data not shown).

The only significant change of body weight was a minimal decrease with captopril (-0.1±0.1 % vs. +0.1±0.1 % with placebo, $P<0.05$). Body weight did not change with either diazoxide or nifedipine (both 0±0.1 %). Hemoglobin decreased slightly on placebo days (-1.1±0.6 %) and comparable changes were observed with diazoxide and captopril (-0.4±0.6 % and -0.1±0.5 %). Only nifedipine induced a significant, but small increase of Hb (+1.7±0.7 %, $P<0.01$ compared to placebo). Parallel to the changes of hemoglobin, serum albumin increased with nifedipine and was not changed significantly by either diazoxide or captopril as compared to placebo (Fig. 3).

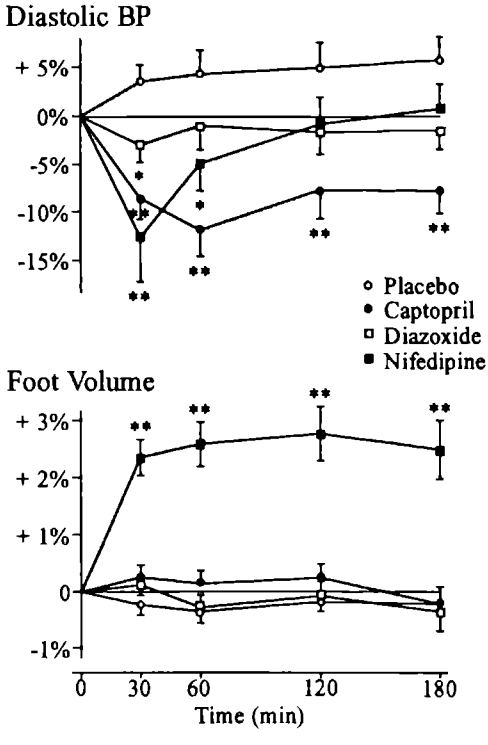


*Figure 2. Relative changes of diastolic blood pressure (BP) and foot volume after placebo and the three active drugs. *$P<0.05$ and **$P<0.01$ compared to placebo (Wilcoxon's rank sum test). Error bars indicate SEM.*

Neither the increase of foot volume nor the increase of natriuresis with nifedipine was linearly correlated to the increase of serum albumin or of hemoglobin with this drug. The increase of foot volume was not correlated to the acute blood pressure lowering effect of nifedipine.

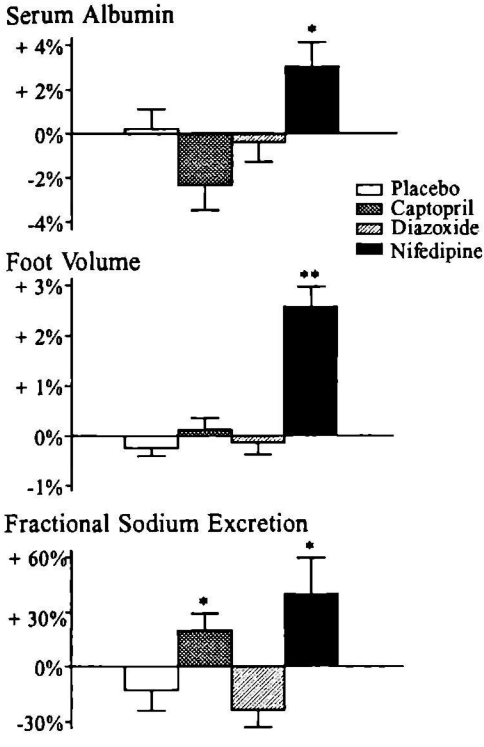


*Figure 3. Mean relative changes of serum albumin, foot volume and fractional sodium excretion (FE_{Na}) after placebo and the three active drugs. *$P<0.05$ compared to placebo (Wilcoxon's rank sum test). Error bars indicate SEM.*

DISCUSSION

In the present study in healthy sedentary volunteers, the CEB nifedipine increased sodium excretion and at the same time induced an acute increase of foot volume, which thus cannot be ascribed to sodium retention. This observation indicates that nifedipine induces a rapid increase of volume in the depending legs by local changes in the peripheral vessels. Our data are thus in line with observations in isolated and denervated cat skeletal muscles where local intra-arterial infusion of different types of CEB induced an acute increase of transcapillary fluid filtration[8]. In this model nifedipine also interfered with the increase in vascular resistance that normally protects capillaries against an orthostatic load[8], which could explain the preference for edema formation in the depending legs. In the human forearm, systemic administration of a dihydropyridine CEB also increased transcapillary fluid filtration[9]. Such increased fluid filtration could be due to an increase of intracapillary hydrostatic pressure following preferential dilation of small precapillary arterioles by CEB[11,12]. On the other hand, experiments in bilaterally nephrectomized rats have suggested that dihydropyridine CEB increase capillary permeability, because nicardipine but not the benzothiazepine diltiazem acutely increased hematocrit more than serum protein[10]. In these rats, nicardipine induced extravasation of Evans blue dye into skeletal muscle which also suggested an increase of capillary permeability for proteins[10].

In contrast to the changes with nifedipine, infusion of the PCO diazoxide was not followed by an increase of foot volume. This difference with nifedipine is

rather unexpected, because CEB and PCO dilate the same precapillary arterioles[12,13] and thus should induce similar increases in intracapillary hydrostatic pressure. It can be questioned, however, whether this dose of diazoxide induced the same degree of peripheral vasodilation as nifedipine, because diazoxide hardly changed blood pressure and did not induce reflex tachycardia. Thus, it is possible that a higher dose of diazoxide would have led to different results. On the other hand, the difference with nifedipine might indicate that different mechanisms underlie edema formation with CEB and PCO, e.g. an increase of capillary permeability with CEB[10] or primary sodium retention with PCO.

The ACE inhibitor captopril lowered blood pressure but, like diazoxide, did not change foot volume. This is not unexpected since ACE inhibitors do not cause edema[14]. These drugs probably do not induce a rise of hydrostatic pressure in the capillaries because of preferential dilation of larger arterioles[15]. Of note, both captopril and nifedipine induced similar increases of natriuresis, but only nifedipine increased hemoglobin and serum albumin. This difference could easily be explained by a nifedipine-mediated redistribution of fluid out of the intravascular compartment into the interstitium as suggested by the foot swelling and in accordance with the aforementioned observations in anephric rats[10].

Since ankle edema with dihydropyridine CEB is the main long-term side effect of these otherwise well-tolerated and highly effective antihypertensive agents[2], elucidation of the mechanism responsible for the edema could lead to successful therapeutic interventions. In a more empirical way, our device might be suited for testing such interventions in short term studies. If our method is used to study the long-term effect of different vasodilators on foot volume, a coefficient of variation of 1.3 % for day to day variability (unpublished observations) should be taken into account.

In conclusion, nifedipine induced parallel increases of foot volume and sodium excretion in healthy volunteers, which supports the hypothesis that the development of ankle edema with dihydropyridine CEB is a local phenomenon and a direct consequence of peripheral vasodilation in the depending legs. In contrast to nifedipine, diazoxide infusion did not induce acute changes of foot volume but also hardly changed blood pressure. This could indicate that diazoxide did not induce enough vasodilation in these normal subjects or that different mechanisms underlie edema formation with PCO and CEB. Further studies are needed to elucidate the mechanisms responsible for the edema formation with PCO.

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CHAPTER 10

General discussion

Calcium entry blockers (CEB) with a dihydropyridine structure such as nifedipine and felodipine are potent arteriolar vasodilators with proven antihypertensive efficacy. Treatment with CEB is largely devoid of the compensatory reactions which have limited the use of other potent vasodilators such as minoxidil. Dihydropyridine CEB induce only a modest, transient activation of the baroreceptor reflex and have a remarkable acute natriuretic effect. These properties make monotherapy feasible and CEB are nowadays one of the first-line choices for patients with hypertension. It is obvious that CEB owe their position for a considerable part to their natriuretic action which prompted us to study this effect in more detail. We have performed several renal clearance studies in healthy volunteers in an attempt to elucidate the mechanisms that are responsible for this natriuretic effect. Thereafter, we studied the natriuretic properties of CEB in hypertensive patients with renal disease, where such an effect might be of specific value. We also addressed the issue whether sodium restriction limits the natriuretic and/or hypotensive effects of CEB in patients with essential hypertension. Finally, we studied the CEB-induced ankle edema which is one of the major side effects of the dihydropyridines and a rather surprising phenomenon in view of the natriuretic effect of these drugs.

Mechanisms of the natriuretic effect of CEB

Renal hemodynamic effects. Early attempts to explain the natriuresis with CEB have concentrated on a possible relation with the renal vasodilating effects of these drugs, as reviewed by Romero[1]. Infusion of low, nonhypotensive doses of vasodilators directly into the renal artery of experimental animals usually increases renal blood flow (RBF) and glomerular filtration rate (GFR). The increase of GFR increases tubular sodium load and the renal vasodilation decreases tubular sodium reabsorption in the proximal tubule or Henle's loop[2]. In this setting, it is not surprising to find an acute natriuresis. However, systemic administration of a vasodilator decreases blood pressure and, consequently, renal perfusion pressure which counteracts an increase of RBF. Under such circumstances, i.e. in the absence of an increase of GFR and RBF, CEB still induce natriuresis in animals[3,4] which strongly supports a direct tubular site of action of these drugs. Micropuncture studies have localized this natriuretic effect of CEB to either the proximal[5] or the distal[6] tubule.

In our studies in healthy volunteers, felodipine infusion never induced consistent changes of GFR in line with most other observations with CEB in man[7]. The natriuretic effect in humans must therefore be the consequence of reduced tubular sodium reabsorption. In the present studies, our indirect estimates of segmental tubular sodium reabsorption indicated decreases of both proximal and distal tubular sodium reabsorption with felodipine confirming previous findings. In all our studies, felodipine, also at a relatively low dose, induced some renal vasodilation, which could explain part of the observed proximal tubular effect. It has been suggested that the CEB-mediated decrease in proximal tubular sodium reabsorption in humans can be ascribed fully to the renal vasodilation, because a very low oral dose of felodipine, which did not induce renal vasodilation, only decreased calculated distal tubular sodium reabsorption[8]. In contrast, however, another dihydropyridine decreased proximal sodium reabsorption at a dose that did not induce any noticeable change of RBF[9]. Therefore, it seems likely that also in humans the distal and at least part of the proximal tubular effects of CEB are explained by factors other than renal hemodynamic changes. The possibility of redistribution of RBF to juxtamedullary nephrons favouring natriuresis cannot be assessed in humans, but is certainly not firmly supported by the available animal data [10,11].

Interaction with sodium retaining hormones. Previously, we demonstrated that felodipine prevented the sodium retention on exogenous angiotensin II (Ang II)[12]. Thus, the question arose whether the natriuretic effect of CEB was merely the consequence of inhibition of the renal sodium retaining effect of endogenous Ang II. In such a case, CEB should no longer induce natriuresis if endogenous Ang II were absent. However, to our disappointment, powerful suppression of endogenous Ang-II production with the angiotensin converting enzyme inhibitor ramipril certainly did not prevent the natriuresis caused by felodipine. Our data did not exclude that ramipril slightly inhibited the natriuretic effect of felodipine and especially its effect on proximal tubular sodium reabsorption, but such an inhibition, if any, was probably due to the blood pressure lowering effect of ramipril. Thus, most of the natriuretic effect of dihydropyridine CEB cannot be ascribed to an interaction with endogenous Ang II. How can these data be reconciled with our previous study, where the interaction between exogenous Ang II and felodipine had been so prominent ? This question prompted us to reevaluate our study with exogenous Ang II. As discussed in chapter 2, the clue for the discrepancy

might be found in the systemic effects of intravenously administered Ang II and especially the rise of systemic blood pressure. This will lead to reflex preglomerular vasoconstriction which could be partly responsible for the sodium retaining effect of exogenous Ang II. Such a reflex myogenic vasoconstriction is very susceptible to CEB. Thus, felodipine might have prevented sodium retention with exogenous Ang II by its effect on preglomerular vasoconstriction and without interfering with the renal tubular effects of Ang II.

Felodipine did not prevent a decrease in urinary sodium excretion induced by infusion of exogenous aldosterone. Hence, it is unlikely that the natriuretic effect of CEB is due to inhibition of the effects of aldosterone. However, since CEB do not counteract the effects of aldosterone, the absence of a rise in aldosterone during treatment with CEB could contribute to their natriuretic effect. Indeed, the natriuretic effect of felodipine appeared to be slightly attenuated in the setting of elevated endogenous aldosterone levels following metoclopramide administration (see following section).

We did not study a possible interaction between norepinephrine and CEB, but the available animal data do not suggest an important role for such an interaction in the natriuretic effect, because CEB did not affect the antinatriuresis induced by low frequency renal nerve stimulation in normotensive rats[13,14].

Distal tubular effects. The natriuretic effect of CEB is, in contrast to most diuretics, not accompanied by an increase in urinary potassium excretion. This beneficial effect could be due to a CEB-mediated inhibition of aldosterone release which is responsible for the generally observed dissociation between an elevated plasma renin activity and unchanged plasma aldosterone concentration (PAC) during treatment with dihydropyridines. Indeed we could clearly demonstrate that infusion of exogenous aldosterone during felodipine not only attenuated the natriuretic effect but was followed by a major increase of potassium excretion. The kaliuretic effect of aldosterone was much more pronounced in the setting of concomitant felodipine infusion than during placebo, which can be explained by a felodipine-mediated increase of sodium and fluid delivery to distal tubular sites. Although these data do not exclude the possibility of another effect of CEB on distal tubular sodium reabsorption, which is the drive for potassium secretion, they certainly indicate that CEB will induce urinary potassium losses if sufficient amounts of aldosterone are present during treatment. The results of this study

could be criticized because of the relatively high, almost supraphysiological PAC, but our observations with metoclopramide and felodipine nicely confirmed our conclusions. Dopaminergic receptor blockade with metoclopramide induces endogenous aldosterone release and in this setting of a physiologic elevation of PAC, infusion of felodipine was followed by an immediate increase of urinary potassium excretion. In our hypertensive patients, however, the elevated PAC after one week of dietary sodium restriction did not lead to kaliuresis (see chapter 7). In this study, PAC rapidly fell to normal levels after administration of felodipine in contrast to our study with metoclopramide. Therefore, PAC has to remain elevated during felodipine in order to induce kaliuresis.

Interaction with intrarenal natriuretic hormones. Selective administration of a CEB into the renal artery increases natriuresis[4]. This suggests that the natriuretic effect could be the consequence of an interaction with intrarenal natriuretic systems such as the dopaminergic and prostaglandin systems, whereas it makes it unlikely that an increased secretion of atrial natriuretic peptide plays a major role. In hypertensive rats, blockade of either the dopaminergic or the prostaglandin system attenuated the natriuresis with CEB[15]. However, very high drug doses were used resulting in very large changes of blood pressure, which makes it hazardous to translate these data to the human situation. Indeed, in our human volunteers, effective dopaminergic receptor blockade with metoclopramide did not affect the natriuresis with felodipine and in another study in humans, the prostaglandin synthetase inhibitor indomethacin did not influence the natriuretic action of oral felodipine[16]. Moreover, in our volunteers, benserazide, a dopa-decarboxylase inhibitor which prevents the intrarenal conversion of inactive dopa to active dopamine also failed to attenuate the natriuresis of felodipine[17]. Taken together, these data make it unlikely that, at least in normotensive humans, these intrarenal natriuretic systems play any role in the natriuretic action of CEB.

Some future perspectives. It is clear from the preceding paragraphs that we are still awaiting a satisfactory explanation for the natriuretic effects of CEB. One of the intriguing questions which remains to be answered is whether the natriuretic action of these chemically heterogeneous drugs can be ascribed to direct inhibition of tubular sodium reabsorption by blockade of the voltage operated calcium channels in tubular cells. The fact that a decrease in

cytosolic calcium, such as induced by CEB, is expected to enhance instead of decrease tubular sodium reabsorption has cast doubt on such a direct link[1]. However, it has been demonstrated recently that TMB-8, an inhibitor of intracellular calcium release, also induced natriuresis[18]. Since most CEB are chiral compounds with the calcium channel blocking ability residing in one of the enantiomers[19], comparison of the natriuretic effects of the two enantiomers might clarify the relation between calcium channel blockade and natriuresis.

If other effects than calcium channel blockade were responsible for the natriuresis with CEB, blockade of renal A_1 adenosine receptors by CEB could be considered because the renal effects of A_1 receptor antagonists[20] show much resemblance with the effects of CEB. Moreover, dihydropyridine CEB, albeit at high dose, can displace A_1 receptor antagonists from their receptors[21].

Natriuretic effects in hypertensive patients

Effects in patients with renal disease. Felodipine infusion induced natriuresis in our hypertensive patients with renal disease, which was quite remarkable in view of the large blood pressure reduction in this study. Thus, CEB also lack sodium retaining effects in this category of patients, which can be difficult to treat and often need a vasodilator. However, the natriuretic effect was smaller than in our normotensive volunteers and appeared to be less with more pronounced renal dysfunction. CEB apparently do not correct the increased sodium and water retention which is directly involved in inducing and sustaining hypertension in many patients with renal disease, especially those with severe renal insufficiency. It is therefore unlikely that CEB per se will reduce the need for diuretics in most of these patients.

Effects at different salt intakes. It has been suggested that CEB reduce blood pressure more effectively at higher salt intakes, which might be ascribed to a larger natriuretic effect. In our outpatients with essential hypertension, felodipine induced a greater acute sodium loss during a salt supplemented diet than during moderate sodium restriction, but this did not lead to a larger hypotensive response. Moreover, although felodipine appeared to neutralize the diet-induced difference in intravascular volume, this again was not translated into a larger reduction of blood pressure during the

salt supplemented diet. We also demonstrated that the diet-induced 5 mm Hg difference in mean arterial pressure was preserved during felodipine and that the lowest blood pressure was reached with felodipine during salt restriction, in line with other studies. The question thus arises whether the reported higher effectiveness of CEB at high salt intake is not merely an artificial phenomenon, because the studies demonstrating it were performed at extremes of salt intake with large differences in blood pressure before administration of the CEB. The much higher blood pressure with high salt intake thus enabled a larger hypotensive response, which is not really surprising since the fall in blood pressure with any agent depends directly on the pretreatment level. Patients are not likely to benefit from such a larger hypotensive response if the final blood pressure remains higher than with a CEB and sodium restriction. Furthermore, the combination of felodipine and high salt intake led to higher foot volumes and visible edema in several patients (see next paragraph). We therefore would not advise unrestricted salt intake during treatment with a CEB.

Edema formation with CEB

In view of the natriuretic effect of CEB, it is surprising that ankle edema is one of the main long-term side effects of dihydropyridines. To study the process of edema formation in more detail, reliable estimates of foot volume were needed. We therefore tested the accuracy of a new simple method, which uses a sensitive balance to measure the force needed for immersion of the foot in a water-bath. The method proved to be accurate with a coefficient of variation of 0.30 % for repeated measurements within a few minutes. Biological changes could be demonstrated easily and were probably responsible for the larger day to day variability of foot volume of 1.31 %. With this method, we studied the acute effects of several vasodilators on foot volume in healthy sedentary volunteers. Diazoxide and captopril did not change foot volume, whereas nifedipine induced an acute increase at the time when urinary sodium excretion was also increased. This increase of foot volume with nifedipine thus cannot be ascribed to sodium retention which supports the hypothesis that ankle edema with CEB is a local phenomenon at the site of vasodilation. Foot volume apparently increases only when an orthostatic load is present, since felodipine did not induce acute changes in our supine hypertensive patients (see chapter 7).

As ankle edema with CEB is not due to sodium retention, it might be speculated that treatment with diuretics and dietary sodium restriction are less effective than in other edematous states. Indeed, in preliminary studies, addition of frusemide to nifedipine did not prevent the acute increase of foot volume in our sedentary volunteers. In apparent contrast, less edema was observed in our hypertensive patients when felodipine was administered during the sodium restricted diet. However, a diet-induced difference in foot volume was already present before the start of felodipine.

It has been suggested that edema formation with a dihydropyridine CEB can be prevented by an angiotensin converting enzyme (ACE) inhibitor[22]. However, in preliminary studies in sedentary volunteers, we observed only a slight insignificant effect of the ACE inhibitor ramipril on the acute increase of foot volume with nifedipine. Further long-term studies in patients are needed to find effective therapeutic measures for the edema with CEB.

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CHAPTER 11

Samenvatting

Voor de behandeling van hoge bloeddruk (hypertensie) zijn tegenwoordig vele soorten geneesmiddelen beschikbaar. Aangezien hypertensie vrijwel steeds gekenmerkt wordt door vernauwing van de weerstandsbloedvaten lijkt het logisch de voorkeur te geven aan de zogenaamde vaatverwijders. De klassieke vaatverwijders hebben echter als nadeel dat zij een compensatoire versnelling van de hartslag en zoutretentie door de nier veroorzaken, waardoor hun effect althans deels teniet gedaan wordt. Calcium instroom remmers (CaIR) zijn een chemisch heterogene groep stoffen die de instroom van calcium in de gladde spiercel van de bloedvaten remmen en zodoende bloedvatverwijding bewerkstelligen. Vooral CaIR met een dihydropyridine structuur zoals nifedipine en felodipine zijn krachtige vaatverwijders die in tegenstelling tot de klassieke vaatverwijders slechts een matige, passagère versnelling van de hartslag veroorzaken. Bovendien geven CaIR geen zoutretentie, maar juist een toegenomen zoutuitscheiding door de nieren (natriurese). Door het grotendeels ontbreken van compensatoire reacties zijn de CaIR één van de eerstelijns keuzen geworden voor de behandeling van hypertensie. In dit proefschrift zijn de natriuretische eigenschappen van dihydropyridine CaIR nader bestudeerd.

Mechanisme van het natriuretische effect van CaIR

Effecten op de nierdoorstroming. CaIR veroorzaken niet alleen verwijding van de weerstandsbloedvaten maar ook van de bloedvaten in de nier. Daardoor kan toediening van een CaIR in de slagader van de nier de nierdoorstroming doen toenemen met natriurese als gevolg. Echter, bij toediening van een CaIR in de aders (intraveneus) of in de vorm van tabletten zal tevens de bloeddruk dalen waardoor de toename van de nierdoorstroming wordt tegengegaan. In dergelijke gevallen veroorzaken CaIR nog steeds natriurese zodat het onwaarschijnlijk is dat bloedvatverwijding in de nier de natriuretische eigenschappen van CaIR volledig kan verklaren.

In overeenstemming met andere onderzoekers vonden wij bij gezonde vrijwilligers na toediening van de CaIR felodipine géén toename van filtratie van bloed in de nier (glomerulusfiltratie) zodat we moeten aannemen dat het natriuretische effect van CaIR berust op verminderde zoutopname (natriumreabsorptie) in de nierbuisjes (tubuli). Verder vonden wij aanwijzingen dat de natriumreabsorptie zowel in het begin (proximaal) als ook aan het einde (distaal) van de tubuli verminderd is. Een deel van het proximale tubulaire effect in onze studies zou verklaard kunnen worden door enige toename van

de nierdoorstroming, die ook bij toediening van een relatief lage dosis felodipine steeds werd waargenomen.

Interactie met natriumretinerende hormonen. Natriumretinerende hormonen zoals angiotensine-II en aldosteron zijn van nature aanwezig en beschermen de mens o.a. tegen overmatig zoutverlies via de nieren. Het natriuretische effect van CaIR zou kunnen berusten op blokkade van de werking van zo'n natriumretinerend hormoon.

In voorgaand onderzoek op onze afdeling is aangetoond dat felodipine de natriumretentie door intraveneus toegediend angiotensine-II voorkomt. Wij hebben derhalve onderzocht of felodipine nog steeds natriurese veroorzaakt als het natuurlijke angiotensine-II onderdrukt wordt door een zogenaamde ACE-remmer, die de omzetting van inactief angiotensine-I in actief angiotensine-II blokkeert. Tegen onze verwachting in bleek de ACE-remmer ramipril het natriuretische effect van felodipine echter niet te voorkomen, zodat het natriuretische effect van CaIR niet verklaard kan worden door een interactie met natuurlijk angiotensine-II. De discrepantie met onze eerder studie kan mogelijk toegeschreven worden aan het bloeddruk verhogende effect van intraveneus toegediend angiotensine-II. Hierdoor kan vernauwing van de bloedvaten in de nier ontstaan die zeer gevoelig is voor CaIR. Zodoende zouden CaIR de natriumretentie door intraveneus angiotensine-II kunnen voorkomen zonder specifieke interactie in de nier.

Felodipine bleek het natriumretinerende effect van intraveneus toegediend aldosteron niet tegen te gaan, waardoor het onwaarschijnlijk is dat het natriuretische effect van CaIR berust op een interactie met natuurlijk aldosteron.

Distale tubulaire effecten. In tegenstelling tot de bevindingen met de meeste plaspillen (diuretica) wordt het natriuretische effect van CaIR niet begeleid door een toename van kaliumuitscheiding. Dit op zich gunstige effect van CaIR zou een gevolg kunnen zijn van remming van aldosteronafgifte uit de bijnier door CaIR. Wij konden inderdaad aantonen dat intraveneus aldosteron niet alleen het natriuretische effect van felodipine remde maar tevens een belangrijke toename van de kaliumuitscheiding veroorzaakte. Deze bevindingen werden vervolgens bevestigd door onze studie met metoclopramide en felodipine. Metoclopramide blokkeert de dopamine receptor (zie volgende paragraaf) in o.a. de bijnier en veroorzaakt daardoor een verhoogde afgifte van natuurlijk aldosteron. Onder deze omstandigheden bleek felodipine ook een toename van de kaliumuitscheiding te bewerkstelli-

gen. Alhoewel het niet uitgesloten is dat CaIR nog andere effecten hebben op de distale natriumreabsorptie en kaliumuitscheiding tonen onze onderzoeken aan dat CaIR kaliumverlies via de nier zullen veroorzaken als er voldoende aldosteron aanwezig is. De remming van aldosteronafgifte uit de bijnier door CaIR draagt derhalve in belangrijke mate bij aan de gunstige combinatie van natriurese zonder toegenomen kaliumuitscheiding tijdens gebruik van CaIR.

Interactie met natriuretische hormonen. Natriuretische hormonen zoals dopamine zijn van nature in de nier aanwezig en dragen bij aan de verhoogde zoutuitscheiding die bijvoorbeeld volgt op een verhoogde zoutinname via de voeding. Het natriuretische effect van CaIR zou kunnen berusten op stimulatie van de dopamine produktie of versterking van het dopamine effect. Blokkade van de dopamine receptor met metoclopramide bleek echter de natriurese door felodipine niet te beïnvloeden, zodat het onwaarschijnlijk is dat het dopamine systeem een belangrijke rol speelt bij de natriuretische werking van CaIR.

Natriuretische effecten bij patienten met hypertensie

Effecten bij hypertensieve patienten met nierziekten. In deze groep patienten wordt de hypertensie veelal veroorzaakt door toegenomen zout- en waterretentie, vooral bij slechte nierfunctie. Felodipine bleek ook bij deze patienten een toename van de zoutuitscheiding te veroorzaken ondanks een gelijktijdige sterke daling van de bloeddruk. CaIR missen dus ook hier het zoutretinerende effect van andere vaatverwijders, hetgeen van groot belang is aangezien dit soort patienten vaak een vaatverwijder nodig heeft voor hun veelal moeilijk te behandelen hypertensie. Het natriuretische effect van felodipine bleek echter geringer dan in onze vrijwilligers, vooral bij patienten met slechte nierfunctie. Het is dan ook onwaarschijnlijk dat CaIR de toegenomen zout- en waterretentie bij deze patienten corrigeren en daarmee de behoefte aan diuretica zouden verminderen.

Effecten bij verschillende zoutinname. Enkele onderzoekers hebben gesuggereerd dat CaIR de bloeddruk sterker doen dalen bij verhoogde zoutinname via de voeding. Een dergelijke eigenschap van CaIR zou toegeschreven kunnen worden aan een sterker natriuretisch effect in deze situatie. In onze poliklinische patienten met hypertensie bleek felodipine weliswaar acuut een groter zoutverlies te geven bij hogere zoutinname, maar dit werd niet gevolgd

door een sterkere bloeddrukdaling. Ook het bloeddrukverlagende effect van een week behandeling met felodipine was vergelijkbaar bij hoge en lage zoutinname. Het gunstige bloeddrukverlagende effect van zoutbeperking bleek aldus behouden te blijven tijdens felodipine zodat de laagste bloeddruk gevonden werd tijdens felodipine en een zoutbeperkt dieet. Bovendien kregen enkele patienten alleen in de periode met hoge zoutinname dikke enkels (oedeem) na het gebruik van felodipine (zie volgende paragraaf). Op basis van deze gegevens zouden wij dan ook willen adviseren om overmatige zoutinname tijdens behandeling met een CaIR te vermijden.

Oedeemvorming tijdens behandeling met CaIR.

In veel gevallen is oedeemvorming aan de enkels een gevolg van toegenomen zout- en vochtretentie door de nieren. Ondanks de evidente natriuretische eigenschappen van CaIR blijkt enkeloedeem verrassend genoeg een van de belangrijkste bijwerkingen van behandeling met dihydropyridine CaIR zoals nifedipine. Om het proces van oedeemvorming nader te bestuderen rees de behoefte aan een betrouwbare maat voor de hoeveelheid oedeem. Wij hebben derhalve een nieuwe, simpele methode voor de bepaling van het voetvolume uitgetest. Hierbij wordt gebruik gemaakt van een gevoelige weegschaal die de kracht meet die nodig is voor onderdompeling van de voet in een waterbak. Deze methode bleek nauwkeurig het voetvolume te bepalen met een variatiecoëfficient van 0.30 % voor herhaalde metingen. Biologische veranderingen van het voetvolume konden gemakkelijk aangetoond worden en waren waarschijnlijk verantwoordelijk voor de grotere dag tot dag variatie van 1.31 %.

In gezonde, zittende vrijwilligers hebben wij met de bovenstaande methode het acute effect van enkele vaatverwijders op het voetvolume bestudeerd. De klassieke vaatverwijder diazoxide had in de door ons gebruikte dosering géén acuut effect op het voetvolume. Nifedipine daarentegen bleek het voetvolume acuut te vergroten in de fase dat er sprake was van een verhoogde zoutuitscheiding door de nier. Deze bevindingen passen bij de veronderstelling dat oedeemvorming tijdens het gebruik van dihydropyridine CaIR berust op een lokaal effect en een direkt gevolg is van de vaatverwijding. In de bovengenoemde patienten met hypertensie bleek oedeemvorming na felodipine weliswaar alleen op te treden in de periode met hoge zoutinname, maar dit hield verband met de verschillen in voetvolume die door het dieet veroorzaakt waren vóór de start van de behandeling met felodipine.

DANKWOORD

De schrijver van een proefschrift zou men kunnen vergelijken met een marathonloper, die weliswaar een lange weg alleen moet afleggen, maar dit slechts succesvol kan volbrengen door de uitgebreide steun van vele anderen. Bij deze wil ik dan ook allen, die hebben bijgedragen aan het tot stand komen van dit proefschrift, van harte bedanken. Zonder iemand tekort te willen doen wil ik met name noemen:

Alle patiënten die bereid waren aan de vaak ingewikkelde onderzoeken deel te nemen en alle proefpersonen die grote hoeveelheden water te verwerken kregen en de voeten koel moesten houden.

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Mijn ouders die mij altijd gestimuleerd hebben om dit te kunnen bereiken.

Ten slotte, Els en kinderen, vooral het laatste jaar zullen jullie ervaren hebben dat het schrijven van een proefschrift bepaald geen sociale gebeurtenis is. Hopelijk breken wat dit betreft nu betere tijden aan.

CURRICULUM VITAE

Henk van Hamersvelt werd op 16 februari 1956 geboren te Rotterdam. Vanaf 1968 bezocht hij in deze stad het Gymnasium Erasmianum, alwaar hij in 1974 het diploma gymnasium-β behaalde. Aansluitend studeerde hij geneeskunde aan de Erasmus Universiteit te Rotterdam. Tijdens deze studie nam hij van 1977 tot 1979 als student-assistent op de afdeling Neuroanatomie (hoofd: Prof. dr. H.G.J.M. Kuypers) deel aan onderzoek naar de motorische besturing van de hand bij de rhesusaap. Het doctoraalexamen werd behaald in oktober 1979 en het artsexamen in september 1981. Aansluitend vervulde hij zijn dienstplicht als kazerne-arts op de Koninklijke Militaire School te Weert. In januari 1983 begon hij zijn opleiding tot internist in het Groot Ziekengasthuis, thans Bosch Medisch Centrum, te 's Hertogenbosch (opleiders: Dr. J.B. Lips en Dr. J.L.J. Jansen). Na juli 1987 werd de opleiding afgerond in het St. Radboudziekenhuis te Nijmegen (opleider: Prof. dr. A. van 't Laar) en op 1 januari 1988 werd hij door de Specialisten Registratie Commissie ingeschreven als internist. Vanaf juli 1987 is hij werkzaam op de afdeling Nierziekten (hoofd: Prof. dr. R.A.P. Koene) van de Kliniek voor Inwendige Ziekten, thans Cluster Inwendige Specialismen, van het St. Radboudziekenhuis te Nijmegen. Aldaar verrichtte hij het onderzoek waarvan dit proefschrift het resultaat is en werd hij opgeleid voor het aandachtsgebied nefrologie binnen de inwendige geneeskunde.

Hij is getrouwd met Els Steinbrück, en vader van Hanneke, Robbert, en Eveline.

Stellingen

behorende bij het proefschrift

Natriuretic Effects of Dihydropyridine Calcium Entry Blockers

Henk van Hamersvelt

17 februari 1994

1. Het natriuretische effect van dihydropyridine calcium instroom remmers kan niet verklaard worden uit een interactie met endogeen angiotensine II of het dopaminerge systeem.

2. Omdat dihydropyridine calcium instroom remmers een stijging van de aldosteronspiegel voorkomen gaat het natriuretische effect van deze middelen niet gepaard met overmatig kaliumverlies via de urine.

3. Mede dankzij hun natriuretische effect zijn calcium instroom remmers een goede keus voor de behandeling van hypertensie bij patiënten met nierinsufficiëntie.

4. Met het oog op de uiteindelijk te bereiken bloeddruk en de kans op oedeemvorming is onbeperkte zoutinname tijdens behandeling met een dihydropyridine calcium instroom remmer af te raden.

5. Oedeemvorming tijdens behandeling met een dihydropyridine calcium instroom remmer berust op een lokaal fenomeen in de afhangende onderste extremiteiten.

6. Gezien de toename van het voetvolume in de loop van de dag is het raadzaam om schoenen pas 's middags aan te schaffen.

7. Bij niertransplantatiepatiënten, bij wie de eigen nieren verwijderd zijn, is het weinig zinvol om een β-blokker te gebruiken voor de behandeling van hypertensie.

8. Bij onbegrepen natriumretentie bij een niertransplantatiepatient dient niet alleen een stenose in de transplantaatarterie maar ook een stenose in de arteria iliaca, proximaal van de anastomose, uitgesloten te worden.

9. "Science is always wrong: it never solves a problem without creating ten more". *(G.B. Shaw, 1856-1950)*

10. De gezinssamenstelling van een promovendus kan men veelal afleiden uit de personen aan wie het proefschrift opgedragen is.

11. Er bestaat waarschijnlijk een sterke positieve correlatie tussen de prijs in een bepaalde winkel en het aantal creditcards waarmee men daar kan betalen.

12. Het opschrift "vertrouwelijk" op verzamelbakken voor oud papier maakt het riskant om werkelijk vertrouwelijke informatie, die vernietigd moet worden, daarin te verzamelen.