Interactions between calcium antagonists and the sympathetic nervous system in various pharmacological models
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Citation for published version (APA):
van der Lee, R. (2000). Interactions between calcium antagonists and the sympathetic nervous system in various pharmacological models

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Chapter 7

COMPARISON OF THE TIME COURSES AND POTENCIES OF THE VASODILATOR EFFECTS OF NIFEDIPINE AND FELODIPINE IN THE HUMAN FOREARM

Submitted for publication
1. Introduction

1,4-Dihydropyridine calcium antagonists (DHP-CA) are currently used as therapeutic agents for hypertension and angina pectoris [1]. The time course of the vasodilator action of CA is an important kinetic property of these therapeutic agents. A too rapid onset of action causes a swift drop in blood pressure leading to the activation of the sympathetic nervous system via the baroreflex system, and hence to a compensatory increase in heart rate [2]. This effect is undesirable in patients with conditions which require CA as therapeutic agents. In 1987 Kleinbloesem et al. observed that a high infusion rate of nifedipine in healthy volunteers leads to sustained reflex tachycardia without a significant decrease in blood pressure. At a slow infusion rate, however, the drug did not provoke tachycardia, whereas a significant and stable decrease in blood pressure occurred [3]. These findings imply that reflex tachycardia is rather triggered by the rate of the vasodilator effect than by its magnitude. The rate of this vasodilator effect is controlled by the rate of administration on the one hand, as shown by Kleinbloesem et al. [3], and also by the application of slow-release preparations now available for nifedipine and other CA [4,5]. On the other hand the time course of the vasodilator effect is also influenced by physicochemical characteristics of the compound, such as for instance the membrane-partition coefficient. This parameter denotes the proportion of substance solved in a lipophilic membrane relative to the proportion in aqueous milieu and it is therefore considered as an accurate measure of lipophilicity [6]. Lipophilicity of CA as an important physicochemical property with regard to the rate of vasodilator effect has been considered, hence the recent development of several new lipophilic DHP-CA.

In an in vitro study using rat small mesenteric arteries we found that there probably exists a relationship between the lipophilicity and the time course of action of various CA [7]. For this reason we thought it of interest to analyse such a possible relationship in vivo in a human vascular bed.

Accordingly, it was the aim of the present study to compare the time courses of two DHP-CA with different degrees of lipophilicity, as calculated by means of the logarithm of the membrane partition coefficient (logP) [8]. Furthermore, we established whether measurements returned to baseline-values within the rigid time boundaries of the experiment. The experiments were performed in a human model as close to the in vitro situation as possible, that is venous occlusion human forearm plethysmography. A major advantage of this model is that an isolated vascular bed can be studied in vivo without the interference of extensive distribution and metabolic phenomena which would occur after systemic administration of the drugs to be studied.
2. Methods

Subjects
Fourteen male healthy non-smoking volunteers (age 31±7 yrs) participated in the present study. A short medical history, physical examination and routine laboratory tests were performed. If no abnormalities were found, subjects were included into the study. Subjects were instructed to refrain from liquorice, drinking alcohol or caffeine-containing beverages at least 12 hours prior to the experiment. Informed consent was obtained and the study protocol was approved by the Medical Ethics Committee of the Academic Medical Center at Amsterdam.

2.2. Experimental Conditions
Each experiment was performed in the morning, with the subject in the supine position, in a quiet room at a temperature of 22-23°C. Forearm and hand volumes were measured by means of water displacement. A one-lead electrocardiogram (ECG) was recorded continuously. After local anesthesia with lidocaine 1%, the brachial artery was cannulated using a XRO Arterial Catheter-Seldinger Technique (Laboratoire Plastimed, Saint-Leu-La-Forêt Cedex, France). The cannula was connected to a Baxter pressure transducer. Drugs were infused into the brachial artery using a B.Braun Secura FT Perfusor (B.Braun, Germany). Both arms were instrumented with mercury-in-silastic strain gauges, which were connected to a Hokanson EC-2 plethysmograph (Hokanson Inc., Issaquah, WA, USA) for the measurement of forearm blood flow (FBF). Heart rate (HR) from ECG, intra-arterial (i.a.) blood pressure, and left and right FBF were recorded on a polygraph (Wekagraph Wk-450-R, Depex bv, De Bilt, The Netherlands). Data were recorded on a personal computer using an analog-to-digital converter (Model DT 2801, Data Translation Inc., Marlborough, MA, USA). Both upper arms were instrumented with pressure cuffs, connected to a Hokanson E-10 rapid cuff inflator. For the measurement of FBF, R-wave triggered cuff inflation (at 40 mmHg) for venous occlusion plethysmography was controlled by the personal computer. FBF was measured 4 times per minute and the mean arterial blood pressure (MAP) was derived from the concomitantly recorded arterial blood pressure. During each infusion experiment the hands were continuously excluded from the circulation by inflating small wrist cuffs to at least 40 mmHg above systolic blood pressure. The infusion experiments were started at least 60 min. after cannulation of the brachial artery. Between the various infusion experiments the wrist cuffs were deflated and sufficient time (at least 30 min.) was allotted to let the subjects recover from hand ischemia and to allow FBF to return to baseline levels.
2.3. Study protocol

The protocol of the study is summarised in Fig. 1. The ischemia-hyperemia protocol was carried out to obtain a measure for the maximal vasodilation to be expected at the highest dose of CA. Values thus obtained were not used for further calculation but as an indication of maximal possible vasodilation in each subject.

The infusion protocol started with the infusion of the substance used to dilute the CA; for nifedipine this was saline (NaCl 0.9%) and for felodipine a solution containing 8% ethanol and 22% polyethylene glycol-400 (PEG-400). Nifedipine was infused in the dosages 0.01, 0.025, 0.1 and 0.25 μg/kg/min, respectively. Felodipine was administered in the dosages 0.005, 0.015, 0.05 and 0.15 μg/kg/min. Infusions were continuous over a period of 20 min. at a standard infusion rate of 0.3ml/min. Before starting any of the infusions, baseline FBF was measured.

2.4. Drugs

Nifedipine (Adalat pro infusione) was purchased from Bayer bv. Mijdrecht, The Netherlands. Felodipine pro infusione was kindly donated by Astra (Astra Hässle AB, Mölndal, Sweden). The recipe for the solvent of felodipine (a solution containing 8% ethanol and 22% polyethylene glycol-400) was provided to the hospital pharmacy, which prepared the solution.

2.5. Data analysis

Since the main topic of investigation was the time course, all measurements were included into the analysis. As a relevant parameter indicating time courses $K_{12}$-values were calculated. For this purpose all values were normalized to percentages and then
analyzed with non-linear regression and a one-site hyperbola based upon the following equation:

\[ Y = \frac{B_{\text{MAX}} \times X}{(K_D + X)}, \]

where \( B_{\text{MAX}} \) is the maximal normalized increase in FBF (by definition 100%) and \( K_D \) is the dissociation constant indicating the time at which half of the maximal vasodilation has occurred.

The percentage change in FBF was calculated relative to the values measured at baseline. The plasma concentration (\( C_{\text{plasma}} \)) in mM of CA was calculated using the equation [9]:

\[ C_{\text{plasma}} = \frac{\text{IR} \times \text{BW} \times 100}{(1 - \text{Ht}) \times \text{FBF} \times \text{V} \times \text{MW}}, \]

where \( \text{IR} \) is the infusion rate, \( \text{BW} \) is the body weight, \( \text{Ht} \) is the hematocrit, \( \text{V} \) is the forearm volume and \( \text{MW} \) is the molecular weight of the CA studied [9]. The average calculated plasma concentrations and the percentage change in FBF were used to construct concentration-response curves by means of a curve fitting computer program (GraphPAD Software, San Diego, CA, USA) based on the relationship:

\[ E = \frac{E_{\text{max}} \times D_p}{(D_p + IC_{50}^p)} , \]

where \( E \) is the effect (% change in FBF), which is achieved at the calculated drug concentration \( D (\log[\text{mol/L}]) \), \( E_{\text{max}} \) is the maximally achievable effect, \( IC_{50} \) is the drug concentration at which 50% of the maximal inhibitory effect is achieved (\( \log[\text{mol/L}] \)), and the exponent \( P \) represents the slope of the relationship (Hill coefficient).

Analyses of variance on the measured values or, where appropriate, t-tests were used to examine effects of the CA on hemodynamics and the time courses of both CA. Results are presented as means ± SEM. A \( P \)-value of less than 0.05 was considered to indicate statistically significant differences.

3. Results

3.1. Hemodynamic parameters
The baseline parameters of the two groups of subjects are given in Table 1. After the last infusion of either of the CA, HR was significantly increased in all subjects. MAP remained unchanged throughout the protocol. FBF did not change in the contralateral arm, suggesting, that no systemic effect of the CA infused had occurred during the experiments (Table 2). Fig. 2 shows a typical computer recording of the change of actual FBF over time. The degree of vasodilation induced by CA clearly showed strong fluctuations over time.
Table 1. Hemodynamic parameters at baseline and at the end of the experiment. Values are given as means ± SEM. n=7 for each CA. * means P<0.05. MAP = mean arterial pressure (mmHg). HR = heart rate (beats/min.).

<table>
<thead>
<tr>
<th></th>
<th>HR baseline</th>
<th>HR end</th>
<th>MAP baseline</th>
<th>MAP end</th>
</tr>
</thead>
<tbody>
<tr>
<td>nifedipine</td>
<td>65 ± 3</td>
<td>78 ± 3*</td>
<td>85 ± 2</td>
<td>88 ± 3</td>
</tr>
<tr>
<td>felodipine</td>
<td>60 ± 2</td>
<td>72 ± 2*</td>
<td>82 ± 5</td>
<td>83 ± 3</td>
</tr>
</tbody>
</table>

3.2 Potency of the CA

Fig. 3 shows the concentration-response curves for the effects of both CA on FBF. In Fig. 4 the logIC50-values are depicted for each individual experiment. The average logIC50-values were -7.46 ± 0.17 and -8.47 ± 0.14 for nifedipine and felodipine, respectively (P<0.001), indicating the higher potency of felodipine over nifedipine. In the felodipine group, three of the seven subjects were very sensitive to the compound, as reflected by a stable, ongoing increase in the baseline of FBF after infusion of the second highest concentration (0.05µg/kg/min), lasting more than 60 min. Because of the risk of systemic effects in these three subjects the highest concentration of felodipine could not be tested.

Table 2. Averaged FBF-values in ml/100ml/min. in the contralateral control arm. No statistical significant differences were found, indicating no systemic effects of the infused CA. Vehicle means measurements during infusion of vehicle in the infusion arm.

<table>
<thead>
<tr>
<th>[nifedipine]</th>
<th>FBF ± SEM</th>
<th>[felodipine]</th>
<th>FBF ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>2.0 ± 0.1</td>
<td>vehicle</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>0.01 µg/kg/min.</td>
<td>2.2 ± 0.1</td>
<td>0.005 µg/kg/min.</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>0.025 µg/kg/min.</td>
<td>2.3 ± 0.2</td>
<td>0.015 µg/kg/min.</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>0.10 µg/kg/min.</td>
<td>2.7 ± 0.4</td>
<td>0.05 µg/kg/min.</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>0.25 µg/kg/min.</td>
<td>3.0 ± 0.4</td>
<td>0.15 µg/kg/min.</td>
<td>3.6 ± 0.9</td>
</tr>
</tbody>
</table>

Table 3. Averaged Kp-values for the time courses of the effects caused by all concentrations of the infused CA. * means significantly different from felodipine 0.15 µg/kg/min (P<0.05).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 µg/kg/min.</td>
<td>5.3 ± 0.7</td>
<td>0.005 µg/kg/min.</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>0.025 µg/kg/min.</td>
<td>5.5 ± 0.6</td>
<td>0.015 µg/kg/min.</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>0.10 µg/kg/min.</td>
<td>3.4 ± 0.6</td>
<td>0.05 µg/kg/min.</td>
<td>5.1 ± 1.0</td>
</tr>
<tr>
<td>0.25 µg/kg/min.</td>
<td>3.1 ± 0.6</td>
<td>0.15 µg/kg/min.</td>
<td>3.4 ± 0.5</td>
</tr>
</tbody>
</table>
Fig. 2. Typical computer recording of the actual change in FBF (ml/100ml/min.) over time. The recording shows the results in a subject infused with the vehicle of felodipine and 0.05 µg/kg/min. of felodipine. Note the fluctuating pattern of the increase in FBF upon infusion with felodipine.

Fig. 3. Concentration-response curves for the vasodilator effect of felodipine and nifedipine on basal forearm blood flow (FBF) in the human forearm.

3.3 Time courses of the vasodilator effects of the CA
In table 3, the averaged $K_D$-values of all the concentrations used are given. The $K_D$-value of the lowest concentration of felodipine (0.005 µg/kg/min) was significantly higher than the $K_D$-value of the highest concentration (0.15 µg/kg/min) ($P<0.05$). This finding reflects the rapid vasodilator effect of felodipine in the highest concentration studied. However, no statistically significant differences were found.
between the $K_D$-values of the effects induced by the various concentrations, or between the time courses of the two CA per se (mean $K_D$-values $4.3 \pm 0.6$ and $4.6 \pm 0.6$ for nifedipine and felodipine, respectively).

![Diagram](image)

Fig. 4. LogIC$_{50}$-values of all individual experiments (n=7 for each group) as calculated for the vasodilator effect of nifedipine (NIF) and felodipine (FEL). Mean IC$_{50}$-values $-7.46 \pm 0.17$ and $-8.47 \pm 0.14$ for nifedipine and felodipine, respectively ($P<0.01$).

4. Discussion

In the present study we quantified the time courses of the vasodilator effects of nifedipine and felodipine in the human forearm vascular bed, expressed as changes in the forearm blood flow. Furthermore, the vasodilator potency of either compound in this specific setting was quantified.

The potencies of both CA differed significantly in this study: $-7.46 \pm 0.17$ and $-8.47 \pm 0.14$ were the logIC$_{50}$-values for nifedipine and felodipine, respectively, indicating the higher potency of felodipine. The $K_D$-values for the highest and lowest concentration of felodipine differed statistically significant from each other, indicating a more rapid vasodilator effect of felodipine in the highest concentration studied. The $K_D$-values for both 1,4-DHP were $4.3 \pm 0.6$ and $4.6 \pm 0.6$ for nifedipine and felodipine, respectively. These values did not differ significantly, indicating that both CA caused vasodilator effects with the same time courses at all concentrations studied.

Perez-Vizcaino et al. (1993) found that felodipine was more potent in inhibiting contractions of single isolated rat aortic smooth muscle cells than nifedipine (pIC$_{25}$, log M $-8.7$ and $-8.5$ for felodipine and nifedipine, respectively) [10]. Similarly, Hagiwara and coworkers (1993) reported maximal inhibition of high K$^+$-induced
contractions with 10 nM felodipine and 100 nM nifedipine in rat aorta [11]. In hypertensive patients, felodipine 5 mg once-daily and nifedipine GITS 30 mg once-daily resulted in a comparable reduction of blood pressure [12]. Accordingly, our finding that felodipine is more potent than nifedipine in the human forearm corresponds with findings in a variety of other models. In contrast, a study in isolated small human arteries in our laboratory revealed that felodipine is equipotent compared with nifedipine [17].

HR proved significantly higher at the end of the experiment when compared with that at baseline, for both CA studied. A direct systemic effects of the DHP resulting in a tachycardic response cannot be fully excluded. However, since there was no rise in baseline forearm blood flow in the contralateral arms of any subject investigated, systemic effects of the CA are rather unlikely. Especially the repeated inflation of the wrist cuffs during experiments is an unpleasant experience, and it should be considered that this rise in HR reflects discomfort of the subjects resulting in a mild sympathetic activation.

Of interest were the clearly fluctuating vasodilator effects as shown in Fig. 2. The two most likely explanations for this phenomenon are effects of breathing on the pulse pressure in the arterial circulation and spontaneous diameter oscillations of the forearm blood vessels itself. Van Bavel et al. (1991) described vasomotion in pressurized rat isolated mesenteric small arteries and the influence of pressure alterations on this phenomenon [13]. Hayoz et al. (1993) reported spontaneous diameter oscillations in the human radial artery using a high-precision A-mode echo-tracking device. They found that sympathovagal mechanisms are not likely to play a role in this phenomenon and that fluctuations in basal tone affect the distensibility of the blood vessels [14].

In our group there exists a vast body of experience with human forearm venous occlusion plethysmography in various areas of pharmacology, including α-adrenergic agonists, serotonin, angiotensin II and AT1-antagonists, calcium antagonists and cholinergic agonists/antagonists [15-18]. Human forearm venous occlusion plethysmography might be considered as an in vivo experiment which closely resembles the in vitro research in isolated (perfused) vessels or isolated perfused vascular beds. This experimental setup allows to study the virtually isolated vascular bed of the human forearm, whereas metabolism is limited to that normally occurring in the vasculature. Despite these similarities with the in vitro situation, the major differences are the presence of blood instead of a physiological buffered solution, and the influences of a beating heart: heart rate and blood pressure.

Studies in vitro, in both rat and human isolated blood vessels, have demonstrated that differences in the time courses between CA (among others nifedipine and felodipine)
appear to be closely related to differences in lipophilicity [7,19]. Moreover, we performed a cross analysis of the time course of nifedipine in three separate projects in our department, including time course studies in rat small mesenteric arteries, human small subcutaneous arteries and the present study. We found that in the case of nifedipine, there was no difference in time course of the effects between the different models, thus indicating that time course is determined by the properties of the drug itself rather than by the experimental method used.

Lipophilicity is quantified by the logarithm of the membrane-partition coefficient (logP) [6]. This value can be estimated using several commercially available computer programs, using the calculation methods developed by Rekker and Mannhold [8]. For nifedipine the calculated logP-value amounts to 2.50 and for felodipine to 4.46, respectively. Consequently, felodipine is approximately 100-fold more lipophilic than nifedipine. Although this difference is more than sufficient to cause significant differences in time courses in the *in vitro* situation, apparently it is different from the human *in vivo* situation as tested in the human forearm [19].

Experimental differences between the present study and the quoted *in vitro* studies also include the method of precontraction which was a high potassium solution in the case of isolated vessel experiments. Furthermore, the isolated vessels were investigated in an isometric wire myograph rather than in a setup of perfused isolated arteries. One or more of such factors may be accountable for the lack of difference in time courses between the two CA investigated as seen in the present study.

In the felodipine group, in three of the subjects the highest concentration could not be administered because of a failure of the FBF to return to baseline. This did not happen in the nifedipine group and this might indicate an effect caused by a higher lipophilicity of felodipine than of nifedipine, thus provoking a longer duration of action.

Experiments with a CA with a high degree of lipophilicity of approximately 6.0 (such as lacidipine or lercanidipine) would have been preferable in the light of this investigation. However, we already found in studies in vitro that such compounds have an excessively long onset and duration of vasodilator activity, which would have been prohibitive in experiments with human volunteers. Furthermore the lipophilicity of such CA would require special solvents which could not have been used in human experiments.

In conclusion, in the present study, felodipine appears to be a more potent vasodilator compound than nifedipine. Only the lowest concentration studied of felodipine induced a vasodilator effect with a significantly higher $K_D$-value than the highest concentration used of felodipine. Apparently, a 100-fold difference in lipophilicity is not enough to generate a substantial difference in time course
between nifedipine and felodipine, as observed in human venous occlusion plethysmography.

References


19. Van der Lee R, Pfaffendorf M, Van Zwieten PA. Differential time courses of various 1,4-dihydropyridine calcium antagonists in relation to lipophilicity in human small subcutaneous arteries. *Submitted for publication*