Interactions between calcium antagonists and the sympathetic nervous system in various pharmacological models
van der Lee, R.

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

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 3

DIFFERENTIAL TIME COURSE OF THE VASODILATOR ACTION OF VARIOUS CALCIUM ANTAGONISTS
1. Introduction

Calcium antagonists (CA) are frequently used in the treatment of hypertension, angina pectoris and supraventricular tachyarrhythmias (verapamil only) [1]. The prototype of the DHP-CA, the non-retarded nifedipine preparation is known to possess several shortcomings. Apart from its negative inotropic activity nifedipine has an unfavourable kinetic profile. Its onset of vasodilator/antihypertensive action is rapid, thus leading to sympathetic activation and reflex tachycardia. Because of its short duration of action it has to be administered 3-4 times daily [2, 3]. Kleinbloesem et al. (1987) demonstrated in healthy volunteers that a high infusion rate of nifedipine leads to a sustained reflex tachycardia without a significant decrease in blood pressure, while at a slow infusion rate the drug did not provoke tachycardia, whereas a significant and stable decrease in blood pressure occurred [4]. These findings imply that reflex tachycardia is rather triggered by the rate of the vasodilator effect than by its magnitude.

Attempts have been made to develop dihydropyridine-CA with a better kinetic profile and less negative inotropic activity than nifedipine. In particular in the series of dihydropyridine-CA (DHP-CA) several new compounds have been introduced and studied clinically. Amlodipine, lacidipine, barmidipine and lercanidipine are examples of newer DHP-CA which are effective antihypertensives without causing substantial reflex tachycardia. Their duration of action is sufficiently prolonged to allow once daily administration in the treatment of hypertension. Most of these newer DHP-CA (lacidipine, barmidipine, lercanidipine) are vasoselective, which implies that they do not depress cardiac contractile force when applied in the usual therapeutic doses [5-8]. Another new CA with a relatively slow onset and long duration of action is mibefradil, chemically related to verapamil [9]. As suggested above, the onset of action of CA, including the newer compounds, appears to be a very important parameter which largely determines the occurrence and severity of sympathetic activation and reflex tachycardia. For this reason we thought it of interest to quantitatively compare the onset of action of a large series of different CA. In table 1 the occurrence of reflex tachycardia in the clinical situation is presented for each of the CA tested [3, 7, 8, 10].

The experiments were performed in isolated vessel preparations in order to exclude the influence of absorption, distribution and other kinetic phenomena which play a role in vivo. The experiments therefore yielded information concerning both the onset and duration of action, as determined by the factors at the cellular level.
Table 1. Occurrence of reflex tachycardia in the clinical situation following oral administration.

<table>
<thead>
<tr>
<th>Calcium antagonist</th>
<th>Reflex tachycardia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nifedipine</td>
<td>Yes</td>
</tr>
<tr>
<td>Verapamil</td>
<td>No</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>No</td>
</tr>
<tr>
<td>Lacidipine</td>
<td>No</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>No</td>
</tr>
<tr>
<td>S,S-barnidipine</td>
<td>No</td>
</tr>
<tr>
<td>Barnidipine HCl</td>
<td>Not tested</td>
</tr>
<tr>
<td>(R)-lercanidipine, (S)-lercanidipine</td>
<td>Clinical as racemic mixture: no</td>
</tr>
</tbody>
</table>

2. Methods

2.1. Isolated small mesenteric arteries
Male Wistar rats weighing 300-350g (Iffa Credo, Les Oncins, France) were anesthetized using a combination of ketamine (120mg/kg i.p.) and xylazine (12mg/kg i.p). Part of the intestine with the adjacent mesenterium was excised and placed immediately in ice-cold Tyrode's solution of the following composition (mM): NaCl 136, KCl 2.5, MgCl₂ 0.5, CaCl₂ 1.8, NaH₂PO₄ 0.42, NaHCO₃ 11.9, glucose 5.5. The adipose tissue surrounding the mesenteric arteries was removed. Before dissection a 40µm diameter stainless steel wire was inserted into the lumen of each vessel to be studied. Subsequently, the vessels were dissected and transferred to the chamber of an isometric wire myograph according to Mulvaney and Halpern (1977) [11]. The preparation was attached to a micrometer screw and, after insertion of a second wire, to an isometric force transducer (Kistler Morse, DSG 6, Redmond, WA, USA). The preparations were equilibrated for 15 min. in Tyrode's solution at 37°C and the medium was oxygenated with carbogen (95% O₂ + 5% CO₂), at a pH of 7.4. Subsequently the vessels were subjected to a normalization procedure according to Mulvaney and Halpern (1976) [12]. The individual circumference was adjusted to 90% of the value the particular vessel would have had at a transmural pressure of 100 mmHg (13.3kPa). Mechanical responses were expressed as active tension, ΔT, that is the developed active force divided by twice the vessel length.

2.2. Experimental protocol
The protocol used is a modification of the protocol as proposed by Videbaek et al. (1995) [13]. Figure 1 is an original registration and shows the different phases in the
protocol. The protocol started with a priming procedure consisting of two contractions induced by a high-potassium Tyrode’s solution (120 mM NaCl was replaced by KCl on an equimolar basis) at intervals of 15 min. A third KCl-induced contraction was allowed to be maintained. After 40 min. of sustained contraction (time point II) one dose of the CA to be studied was added to the organ bath and evaluated for 120 min. In this time interval, the first 20 minutes were divided into four segments of 5 min.; measurements were made at 40, 45, 50, 55 and 60 min. During the remaining 100 min. the relaxing effect of the CA tested was measured at intervals of 20 minutes. Subsequently the medium was replaced by normal, CA-free Tyrode’s solution (time point III). After 20 min. a series of four KCl-induced contractions of 5 min. duration and with intervals of 20 min., were applied to evaluate the duration of action of the CA tested after removal of the CA from the organ bath (recovery). We validated our protocol by control experiments using the same protocol without CA. When relaxation was achieved at equilibrium the corresponding decrease in force was expressed as a percentage of the basal value (time point II = 100%) and taken as the $E_{eq}$. This was done for all concentrations. These values were analyzed to obtain concentration-response curves (CRC) for all CA studied. The time required to achieve the maximal relaxation ($E_{eq}$) was quantified. IC$_{50}$-values were calculated using the constructed CRC’s. The time to $E_{eq}$-values were used to estimate the time to $E_{eq}$ of the calculated IC$_{50}$-concentrations. Recovery was determined as follows: time point I (figure 1) was set at 100%, this because the recovery should return to baseline conditions without taking into account the possible effects of the maintained KCl-induced contraction. Maximal recovery was reached at the fourth KCl-induced contraction at time point IV. Recovery was then calculated as a ratio of time point IV and time point I. At the time points I and IV the medium in the organ bath is CA-free.
2.3. Statistical evaluation

Unless stated otherwise the values in the text are given as means ± standard error of the mean (SEM). Statistical significance was evaluated by one-way ANOVA, followed by Newman-Keuls' test for multiple comparisons, or Student's t-test when appropriate. The level of significance was set at p<0.05.

2.4. Calculations

Using a computer program (GraphPad Prism, GraphPad, San Diego, USA) all curves were fitted to log concentration-effect data for 4-10 individual experiments. The underlying equation is $E=E_{eq} \cdot (A^{p} + IC_{50}^{p})^{-1}$. In this equation E is the response obtained at a given concentration A, $E_{eq}$ is the maximally attainable response, $IC_{50}$ the concentration for the half maximal effect, and the exponent p describes the slope of the relationship (Hill-coefficient). Curves were fitted to averaged concentration-effect data.

2.5. Drugs used

The following drugs were used: ketamine (Nimatek®, Eurovet, Bladel, The Netherlands); xylazine (Rompun®, Bayer bv, Leverkusen, Germany); nifedipine HCl and verapamil HCl (Sigma Chemical Co., St. Louis, MO, USA), mibebradil (Hoffman-LaRoche, Basel, Switzerland), diastereomer barnidipine HCl (S,S: R,R: R,S: S,R in a 33:33:17:17 ratio).

Figure 1. Original recording (barnidipine HCl, 1nM) showing the different phases in the protocol. I=reference point for measurement of recovery; II= adding of one concentration of the CA to be tested; III= end of the sustained contraction, wash-out; IV= measurement point for recovery. Experiments in rat small isolated mesenteric artery preparations.
46

Time course of action of calcium antagonists

and its single enantiomer S,S-barnidipine (Yamanouchi Europe, Leiderdorp, The Netherlands), (S)- and (R)-lercanidipine (Recordati, Milano, Italy), lacidipine (Boehringer Ingelheim, Alkmaar, The Netherlands) and amlodipine (Pfizer, USA). Verapamil and mibefradil were dissolved and further diluted in distilled water; nifedipine, barnidipine HCl, S,S-barnidipine, (S)- and (R)-lercanidipine, lacidipine and amlodipine were first dissolved in 67% dimethyl sulfoxide (DMSO) and the solutions were then further diluted with distilled water. Experiments with all dihydropyridines were performed under the exclusion of light.

3. Results

3.1. Isolated small mesenteric arteries; basal parameters
The mean normalized diameter of the vessels amounted to 256±3µm and the mean active force developed was 2.3 ± 0.1 mN/mm. This classifies the vessels used as resistance arteries. In control experiments (n=10) a stable response could be obtained with a sustained KCl-induced contraction for at least three hours duration: recovery from this sustained contraction was 99.2 ± 2.9% and thus complete.

3.2. Potency of CA as vasorelaxant drugs
Two examples (amlodipine and mibefradil) of a CRC are presented in figure 2. As expected, mibefradil and verapamil appeared to be the least potent vasorelaxant agent studied, whereas S,S-barnidipine proved the most potent vasorelaxant agent tested (figure 3); in the order of potency: S,S-barnidipine > (S)-lercanidipine > barnidipine HCl > Amlodipine > nifedipine = lacidipine > (R)-lercanidipine > verapamil = mibefradil. Interestingly, the diastereomer barnidipine HCl was 20 times less active than the single enantiomere S.S-barnidipine; this difference was highly significant (p<0.005. unpaired t-test).
3.3. Time course of the relaxant effect; time to $E_{eq}$ ($IC_{50}$-concentration)

Lacidipine displays the slowest onset of relaxant action compared to the other CA tested. Both enantiomers of lercanidipine, S,S-barnidipine and barnidipine HCl demonstrate a time to $E_{eq}$ between 45 and 65 min. and statistically they do not differ from each other. As to be expected, nifedipine and verapamil display a rapid onset of action. Mibefradil seems to display a slower onset of action than nifedipine and verapamil, but this difference did not reach statistical significance (figure 4). Accordingly, the onset of action (based on $E_{eq}$-values) adheres to the following sequence: lacidipine = amlodipine > (S)- and (R)-lercanidipine, S,S-barnidipine, barnidipine HCl > mibefradil, verapamil, nifedipine.
3.4. Time course of the relaxant effect; recovery
After wash-out, recovery from nifedipine amounts to 102% and must be considered as complete. Recovery from verapamil and S,S-barnidipine is relatively high, reaching values of 84% and 83%, respectively, whereas recovery from (S)-lercanidipine is very low: 24% (figure 5). With respect to the recovery the following rank order holds for the CA studied: nifedipine > verapamil, S,S-barnidipine, amlodipine > barnidipineHCl, lacidipine > mibefradil, (R)-lercanidipine > (S)-lercanidipine. An overview of the data regarding the IC₅₀-values, time to E₀ (% IC₅₀-concentrations) and recovery are given in table 2 for all CA tested.
**Time course of action of calcium antagonists**

**Figure 4.** Bar chart of time to $E_{eq}$ for $IC_{50}$-concentrations ± SEM for all CA tested in ascending order. Note that lacidipine does not have an error bar, due to the fact that $E_{eq}$ was not reached within 120 min. For statistical data see table 1. For abbreviations see legend Figure 3.

**Figure 5.** Bar chart showing the mean recovery ± SEM for all CA tested in descending order. Values are calculated as ratio of the value at 5 min. of the fourth KCl-induced contraction (time point IV, figure 1) and time point I (figure 1). For statistical data see table 1. For abbreviations see legend Figure 3.
Table 2. Overview table of all data ± SEM collected in this study. For each CA tested n=6. Means with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Calcium antagonist</th>
<th>IC$_{50}$ (nM)</th>
<th>Time to E$_{max}$ (min.)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nifedipine</td>
<td>6.6 ± 0.1</td>
<td>A</td>
<td>102.2 ± 1.1</td>
</tr>
<tr>
<td>Verapamil</td>
<td>30.7 ± 8.6</td>
<td>B,C</td>
<td>84.3 ± 2.6</td>
</tr>
<tr>
<td>Mibefradil</td>
<td>38.6 ± 5.8</td>
<td>B</td>
<td>57.2 ± 3.1</td>
</tr>
<tr>
<td>Lacidipine</td>
<td>6.4 ± 0.9*</td>
<td>A</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>2.0 ± 0.3</td>
<td>D</td>
<td>78.9 ± 4.7</td>
</tr>
<tr>
<td>S,S-barnidipine</td>
<td>0.01 ± 0.002</td>
<td>E</td>
<td>82.8 ± 1.6</td>
</tr>
<tr>
<td>Barnidipine HCl</td>
<td>0.2 ± 0.03</td>
<td>F</td>
<td>70.9 ± 6.0</td>
</tr>
<tr>
<td>(R)-lercanidipine</td>
<td>17.1 ± 3.1</td>
<td>C</td>
<td>47.5 ± 3.1</td>
</tr>
<tr>
<td>(S)-lercanidipine</td>
<td>0.09 ± 0.02</td>
<td>G</td>
<td>23.5 ± 2.2</td>
</tr>
</tbody>
</table>

* IC$_{50}$-value of lacidipine is an estimate.

4. Discussion

The method used in this study allows a quantitative comparison between CA with respect to their vasodilator action at the cellular level. Excluding influences which occur in vivo, like absorption and distribution (e.g. into membranes, lipid structures, proteins and blood cells), in this model the vasodilator effects resulting from the direct interaction of the CA with the L-type calcium channel can be studied and compared quantitatively.

In this study three parameters concerning the vasodilator effect of the CA have been studied: potency (given as IC$_{50}$-values), time of onset of action and duration of action measured as recovery of the contractile response of the vessels after wash-out. The following order for the potency of the CA studied was found: S,S-barnidipine > (S)-lercanidipine > barnidipine HCl > amlodipine > nifedipine = lacidipine > (R)-lercanidipine > verapamil = mibefradil. The time of onset of action adhered to the following sequence: lacidipine = amlodipine > (S)- and (R)-lercanidipine. S,S-barnidipine, barnidipine HCl > mibefradil, verapamil, nifedipine. The rank order for the recovery is as follows: nifedipine > verapamil, S,S-barnidipine, amlodipine > barnidipine HCl, lacidipine > mibefradil, (R)-lercanidipine > (S)-lercanidipine.

An interesting finding in this study is the fact that S,S-barnidipine alone is twenty times more potent than the diastereomer barnidipine HCl: this might indicate that one or more
of the other enantiomers actually have an inhibiting effect on the effects of the S,S-enantiomer. Further studies of the other enantiomers (alone and in all possible combinations) should clarify this issue.

The newer DHP are a rather heterogeneous group of compounds, which differ in side chain composition and their pKₐ, lipophilicity and binding affinity for the receptor [2]. This heterogeneity might well be related to the variety in time courses of the compounds investigated.

Lacidipine, the CA with the slowest onset of action, appeared to have a time course exceeding the boundaries of our protocol, thus confirming the findings of Pfaffendorf et al. (1993) who observed that the vasodilator effect of lacidipine in rat isolated coronary arteries requires at least 5 hours to reach its equilibrium [14]. Accordingly, the concentrations of lacidipine as used in the present study are too high to calculate an accurate IC₅₀-value comparable to literature. Most dihydropyridines (DHP) and especially the newer compounds, are very lipophilic. This results, for instance with lacidipine, in a three compartment kinetic action and a high membrane partition coefficient; the consequences of this property are a slow onset of action and a long duration of action. Differences in membrane partition coefficient and lipophilicity could be hypothesized to account for the differences observed between the DHP [15].

Amlodipine, one of the newer DHP, however, is less lipophilic and possesses a tertiary amino side-chain with a pKₐ of 8.6; at pH=7.4 this side-chain is protonized (and thus positively charged) and may thus interact electrostatically with the negatively charged region of the phospholipid head group of the membrane. This interaction may slow the diffusion of the molecule through the membrane to its receptor site, in addition to keeping the molecule at its receptor site once it is bound. Accordingly, this structural characteristic of amlodipine may account for its slow onset and offset of action and association and dissociation kinetics [5].

Mibefradil has a stronger affinity for T-type calcium channels (TCC) than for L-type calcium channels (LCC), although its effects on LCC is sufficient to account for its vasodilator properties [16]. However, its onset of action in vitro appears to be rather rapid when compared with its effects observed in patients: a decrease in plasma noradrenaline, negative chronotropic activity and no occurrence of reflex tachycardia during the dosing interval. Recently, Göthert et al. (1997) demonstrated that mibefradil also blocks N-type calcium channels. In therapeutic concentrations these channels are known to be involved when noradrenaline-release is triggered as a result of sympathetic nervous system activation [17]. This phenomenon may account for the ability of mibefradil to decrease the plasma noradrenaline level and sympathetic activity. In
addition TCC are thought to be involved in the conductive system of the heart and their blockade by mibefradil may contribute to the drug's negative chronotropic and dromotrophic activities [18].

Unexpected was the finding that the recovery of S,S-barnidipine is more than 80%, in spite of its highly lipophilic character. A high dissociation ratio, either due to stereochemical or electrochemical properties might be responsible for this effect.

The low recovery of both enantiomers of lercanidipine (but especially of (S)-lercanidipine) suggests a long duration of action in vivo. Indeed, Ambrosioni et al. (1997) showed that a single dose (20mg) of lercanidipine in patients with mild to moderate essential hypertension provided a decrease in blood pressure which persists for 24 hours [10].

The differences in responses obtained for the recovery can not be explained entirely by differences in lipophilicity or membrane partition coefficients. Lacidipine and lercanidipine are known to have similar membrane partition coefficients, but the difference in recovery of both CA is highly significant [6]. This suggests that the vasodilator activity of CA is the sum of different properties rather than just the interaction between CA and the L-type calcium channel.

Apart from the absence of reflex tachycardia, the newer CA display other advantages over the old CA such as non retarded nifedipine. The long duration of action of these compounds, as shown in this study as well as in numerous clinical studies, allows a once-daily dosing schedule, thus improving the patients' compliance. Moreover, the combination of a slow onset of action and long duration of action will result in a more stable decrease in blood pressure and a trough to peak ratio greater than 0.5, as required in the guidelines issued by the United States Food and Drugs Administration [19].

In summary, vasoselective CA (DHP) which display a slow onset of action in vitro do not cause reflex tachycardia in hypertensive patients. But, as the examples of mibefradil and verapamil show, other mechanisms may also be involved in the prevention of this unwanted side-effect.

References

1. van Zwieten PA, Pfaffendorf M. Similarities and differences between calcium antagonists: pharmacological aspects. *J Hypertens* 1993; 11 (suppl. 1): S3-S11
2. Meredith PA, Reid JL. Differences between calcium antagonists: duration of action and trough to peak ratio. *J Hypertens* 1993; 11 (suppl. 1): S21-S26
10. Ambrosioni E, Circo A. Activity of lercanidipine administered in single and repeated doses once daily as monitored over 24 hours in patients with mild to moderate essential hypertension. *J Cardiovasc Pharmacol* 1997; 29(Suppl.2): S16-S20