Interactions between calcium antagonists and the sympathetic nervous system in various pharmacological models
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Chapter 4

THE DIFFERENTIAL TIME COURSES OF THE VASODILATOR EFFECTS OF VARIOUS 1,4-DIHYDROPYRIDINES IN ISOLATED HUMAN SMALL ARTERIES ARE CORRELATED TO THEIR LIPOPHILICITY
1. Introduction

Calcium antagonists (CA) are frequently used in the treatment of hypertension, angina pectoris and supraventricular tachy-arrhythmias (verapamil only) (van Zwieten and Pfaffendorf, 1993) [1]. The prototype of the 1,4-dihydropyridine (DHP) CA, the non-retarded nifedipine preparation is known to display several shortcomings. Apart from its slightly 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 (Meredith and Reid, 1993; Michalewicz and Messerli, 1997) [2,3], unless it is administered as a slow release preparation.

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. In contrast, at a slow infusion rate the drug did not provoke tachycardia, whereas a significant and stable decrease in blood pressure occurred. These findings imply that reflex tachycardia is triggered by the rate of the vasodilator effect [4].

The rate of this vasodilator effect is controlled by the rate of administration on the one hand, as shown by Kleinbloesem et al. for the slow-release preparations of nifedipine available at present. On the other hand the rate of onset is probably governed by physicochemical characteristics of the active compound, such as the membrane-partition coefficient. This parameter, expressed as logP, denotes the proportion of substance solved in a lipophilic membrane relative to the amount in aqueous milieu and thus reflects lipophilicity. Lipophilicity of CA as an important kinetic property with regard to the rate of vasodilator effect has been considered for some time, and it is reflected by the high logP-values of newly developed 1,4-dihydropyridine (DHP) CA, such as lacidipine and lercanidipine. Reflex tachycardia is not seen after administration of these compounds. Interestingly, in patients taking CA in oral formulations, T\textsubscript{max} (time necessary to reach peak plasma concentrations) is comparable for both lipophilic compounds (such as lacidipine and lercanidipine) and for nifedipine. This is in contrast with their duration of action in patients: lipophilic CA need only once-daily dosing to reach trough to peak ratio's greater than 0.5. Accordingly, lipophilicity of a CA might well be the major parameter governing time of action of a CA. In an in vitro study using rat small mesenteric arteries we found that there probably exists a relationship between lipophilicity and the time course of action [5]. This finding prompted us to extend our observations to studies with human vessels in vitro.
Felodipine 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. Felodipine and lercanidipine are vasoselective, which implies that they do not depress cardiac contractile force when applied in the usual therapeutic vasodilator doses (Lund-Johansen, 1990; Herbette et al., 1997) [6,7]. As suggested above, the onset of action of CA 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 vasodilator action of three DHP-CA, nifedipine, felodipine and S-lercanidipine. (S)-Lercanidipine is the most active of the two enantiomers present in the racemate of this drug when used clinically by means of tablets. We also explored a possible relationship with between the lipophilicity of the various compounds (expressed by their logP-values), and the time courses of their vasodilator actions.

The experiments were performed in isolated human 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 about the onset of action, as determined by the factors at the cellular level.

2. Methods

2.1. Vessel preparations
Vessels were obtained from subcutaneous fat derived from cosmetic surgery (mamma reduction and abdominoplasty) in healthy women (age range 18-45 yrs) who, to our knowledge, did not take any medication apart from oral contraceptives. Arteries and veins were distinguished by means of their morphometric differences in the larger vessels. Side branches of the larger arteries were dissected. Before dissection a 40μm diameter stainless steel wire was inserted into the lumen of each vessel to be studied. The vessels were excised and placed immediately in ice-cold University of Wisconsin solution (UW) with the following composition: Na (mM): Na⁺ 29, K⁺ 125, Mg²⁺ 5, SO₄²⁻ 5, adenosine 5, lactone bionic acid 100, raffinose 30, allopurinol 1, glutathion 3, hydroxyethyl starch 50g/L, dexamethasone 16 mg/L, insulin 40 U/L and penicillin G 200,000 U/L. The vessels were stored in UW for a period of 12-48h at 4°C. The preparations were transferred to the chamber of an isometric wire myograph according to Mulvany and Halpern (1977) [8] containing 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 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 Mulvany and Halpern (1976) [9]. 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) [10]. The original recording depicted in Fig.1 shows the different phases of 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 20 min. Subsequently a contraction induced by phenylephrine (3 μM) followed by relaxation caused by methacholine (3 μM) was observed in order to test the α-receptor-mediated responses and the relaxation by endothelium-mediated mechanisms, respectively. Vessels displaying less than 80% relaxation to methacholine were discarded. A KCl-induced contraction was then provoked, followed by a fourth KCl-induced contraction which was allowed to persist. After 40 min. of sustained contraction one dose of the CA to be studied was added to the organ bath and evaluated for 120 min. We validated our protocol by control experiments using the same protocol without CA. IC₅₀-values were calculated using the maximal responses obtained for each concentration of the CA tested. Time to Eₑ₀ was calculated using the time-values consistent with 90% of the maximal response of each concentration of the CA tested.

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. Data analysis
Using a computer program (GraphPad Prism, GraphPad, San Diego, USA) all curves were fitted to log concentration-effect data for individual experiments (n=7). The underlying equation is E=Eₓₘₐₓ\text{A}^\text{p}(\text{A}^\text{p} + \text{IC}_{₅₀}^\text{p})^{-1}. In this equation E is the response obtained at a given concentration A. Eₓₘₐₓ is the maximally attainable response, IC₅₀
the concentration for the half maximal effect, and the exponent $p$ describes the slope of the relationship (Hill-coefficient). A possible correlation between logP and mean time to equilibrium effect ($E_{eq}$) was analyzed using linear regression analysis including calculations of the 95% confidence interval.

Fig.1. Experiments with isolated human subcutaneous blood vessels. Original recording of an experiment, showing the various phases in the protocol. Contractions to high potassium (KCl, 120 mM) and phenylephrine (Phe, 3μM) and relaxations to metacholine (MCh, ) and calcium antagonists (CA). As an example the vasodilator effect of felodipine 0.1 μM is shown.

2.5. Drugs used
The following drugs were used: nifedipine HCl, phenylephrine and metacholine (Sigma Chemical Co., St. Louis, MO, USA), felodipine (Astra Hässle AB, Mölndal, Sweden) and (S)-lercanidipine (Recordati, Milano, Italy). Phenylephrine and metacholine were dissolved and further diluted with distilled water; nifedipine, felodipine and (S)-lercanidipine were first dissolved in 67% dimethyl sulfoxide (DMSO) and the solutions were then further diluted with distilled water. All experiments were performed under the exclusion of light.

3. Results

3.1. Isolated human small subcutaneous arteries; preparation
The dissected vessels did not appropriately react to KCl, directly after preparation. Storage in UW was necessary for at least 12 hours to ensure appropriate vessel
reactivity to stimuli, such as high potassium and phenylephrine. All vessels were studied within 48 hours of dissection.

3.2. Isolated human small subcutaneous arteries; basal parameters
The mean normalized diameter of the vessels amounted to 591 ± 52μm and the mean active force developed was 2.7 ± 0.5 mN/mm. Accordingly, the vessels used might be considered as resistance arteries. In control experiments (n=6) a stable response could be obtained with a sustained KCl-induced contraction for at least three hours duration.

3.3. Potency of CA as vasorelaxant drugs
The CRC’s of the three CA tested are presented in figure 2. Apparently, in isolated human resistance arteries, nifedipine, felodipine and (S)-lercanidipine showed the same potency which is reflected by their respective logIC$_{50}$-values: -8.46 ± 0.09, -8.33 ± 0.25 and -8.72 ± 0.16 for nifedipine, felodipine and (S)-lercanidipine, respectively (n.s.).

![Fig.2](image-url)

Fig.2. Concentration-response curves (CRC) with respect to the vasodilator effect of all three CA investigated. In human isolated subcutaneous small arteries the CRC’s appear to be similar for all three CA tested.
Fig. 3. Individual logIC₅₀-values for all CA tested. Mean logIC₅₀-values were -8.46 ± 0.09, -8.33 ± 0.25 and -8.72 ± 0.16 for nifedipine (NIF), felodipine (FEL) and (S)-lercanidipine (LER), respectively. There is no significant difference between the calculated values.

3.4. Time course of the relaxant effect; time to $E_{eq90}$
Table 1. shows the values for time to $E_{eq90}$ for all concentrations of the CA tested. Nifedipine displays a similar time to $E_{eq90}$-value for all concentrations studied. Felodipine and (S)-lercanidipine both show statistically significant differences between the values of the two lower concentrations ($10^{-10}$ and $10^{-9}$) and the higher concentrations ($10^{-8}$ and $10^{-7}$). Overall, (S)-lercanidipine displayed the slowest onset of relaxant action when compared to the other CA tested. As to be expected, nifedipine displayed a rapid onset of action. The onset of action of felodipine is intermediate between those of nifedipine and (S)-lercanidipine. Accordingly, the onset of action (based on $E_{eq90}$-values) adheres to the following sequence: (S)-lercanidipine >felodipine>nifedipine.

Table 1. Mean values for all concentrations of CA tested for the Time to $E_{eq}$ (min. ± SEM).

<table>
<thead>
<tr>
<th>log M concentration</th>
<th>Time to $E_{eq}$ (min.) (nifedipine)</th>
<th>Time to $E_{eq}$ (min.) (felodipine)</th>
<th>Time to $E_{eq}$ (min.) ((S)-lercanidipine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td>10 ± 1</td>
<td>77 ± 2</td>
<td>116 ± 4</td>
</tr>
<tr>
<td>-9</td>
<td>10 ± 1</td>
<td>67 ± 7</td>
<td>108 ± 6</td>
</tr>
<tr>
<td>-8</td>
<td>11 ± 1</td>
<td>37 ± 7*</td>
<td>81 ± 10*</td>
</tr>
<tr>
<td>-7</td>
<td>7 ± 2</td>
<td>37 ± 9*</td>
<td>6*</td>
</tr>
</tbody>
</table>

* means significantly different compared to log M concentration -10 and -9 of felodipine and (S)-lercanidipine, respectively ($P<0.05$).
3.5. Analysis of a possible correlation between logP-values and onset of action
(time to \(E_{eq90}\))

Fig. 4 shows the linear regression analysis as performed to analyze the correlation
between logP and mean time to \(E_{eq90}\). Goodness of fit expressed by \(r^2\) was 0.99, and
the slope of the regression line was statistically significant from zero (\(P<0.05\)),
indicating that the correlation between the two parameters is highly significant.

![Graph showing linear regression analysis]

Fig. 4. Linear regression analysis of the correlation between the logP and mean time to equilibrium
effect (\(E_{eq}\)). The correlation proved to be linear and significant. Dotted lines are the calculated 95% confidence limits.

4. Discussion

The method used in the present study allows a quantitative comparison between CA
with respect to their vasodilator actions at the cellular level. 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, since various factors
which are present in vivo (e.g., absorption and distribution, metabolism, elimination,
proteins and blood cells), are ruled out in vitro.

Human subcutaneous arteries have previously been used successfully to study
cardiovascular drug effects [11-13]. In the present study two parameters concerning
the vasodilator effect of the CA have been established: potency (given as \(\text{logIC}_{50}\)-
values) and time course of action. The three 1,4-dihydropyridine CA studied
appeared equipotent with regard to their vasodilator effect: \(\text{logIC}_{50}\)-values were
-8.46 ± 0.09, -8.33 ± 0.25 and -8.72 ± 0.16 for nifedipine, felodipine and (S)-
lercanidipine, respectively (n.s.). However, the time of onset of action was
significantly different and adhered to the following sequence: (S)-lercanidipine>felodipine>nifedipine. The DHP-group is rather heterogeneous, the CA within this category largely differ with respect to side chain composition, pKₐ-values, lipophilicity and binding affinity for the receptor (Meredith and Reid, 1993)[14]. This heterogeneity might well be related to the variety in time courses of the compounds investigated. Felodipine and (S)-lercanidipine belong to the newer agents of the DHP-category.

Most dihydropyridines (DHP) and in particular the newer compounds, are clearly lipophilic. This property results, for instance for (S)-lercanidipine, in a three compartment kinetic profile and a high membrane partition coefficient. Herbette et al. (1993) showed that lacidipine (which has a membrane partition coefficient of 103,000 at a cholesterol:phospholipid ratio of 0.3:1) slowly partitions in and out of cellular membranes [15]. A lipophilic compound readily dissolves in cellular membranes upon entering the body and hereby depots are formed which will release the compound slowly over time. A slow onset and a long duration of action are the most likely consequences of the characteristics and mechanisms described above. Differences in membrane partition coefficient and consequently, lipophilicity, could thus be hypothesized to account for the differences in time to E\textsubscript{eq} observed between the DHP studied. Lipophilicity is quantified by the logarithm of the membrane-partition coefficient (logP) [16]. This value can be estimated using several commercially available computer programs, using the calculation methods developed by Rekker and Mannhold [17]. The logP-values calculated for the three CA investigated, amounted to 2.50, 4.46, and 6.88 for nifedipine, felodipine and (S)-lercanidipine, respectively. A higher logP-value indicates a higher degree of lipophilicity. In the present study, linear regression analysis proved that a slower onset of action as indicated by a higher time to E\textsubscript{eq} correlated with a higher lipophilicity of the compound tested. Accordingly, for the three compounds tested the physicochemical property lipophilicity appears to be a major factor in determining their onset of action.

The fact that various DHP-CA, when administered as oral preparations (except the retarded formulations) reach their peak plasma concentrations in a comparable time (T\textsubscript{max}), supports the hypothesis that the time course of action of a CA is determined within the effect compartment itself, i.e. the plasma membrane. Extrapolated to the in vivo situation, a high lipophilicity of CA results in a slow onset of action, implying that reflexstachycardia, as commonly seen after the administration of non-retarded nifedipine, does not occur. 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 higher than 0.5, as
required in the guidelines issued by the United States Food and Drugs Administration (Rose and McMahon, 1990) [18].
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 without accompanying reflex tachycardia [19]. In a study by Goldsmith (1995), investigating the effect of felodipine and amiodipine on sympathetic activity and baroreflex function in normal humans, felodipine did not alter sympathetic activity or sensitize the baroreflex function after oral administration of the drug [20]. However, in another study felodipine produced a mild increase in heart rate upon oral administration in patients with essential hypertension (Muir et al., 1985) [21].
In summary, 1,4-DHP-CA with a high degree of lipophilicity display a slow onset of action in vitro. The correlation between the logP-values and time to $E_{eq90}$ is highly significant, suggesting a direct relationship between the two parameters. We conclude that the logP-value of 1,4-dihydropyridine calcium antagonists is a predictive parameter for the time of onset in vitro. CA of which do not require extended-release preparations to prolong time of onset and duration of action generally have a logP-value of approximately 6.0 (lacidipine, manidipine). Consequently, when applied to the in vivo situation, logP-values higher than 6.0 are required to increase the time of onset of vasodilator action of calcium antagonists to such a level that reflex tachycardia does not occur.

References

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