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Time course of action of amlodipine and felodipine in the rat is most rapid in small arteries

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Abstract

The time course of action of amlodipine was compared to that of felodipine in rat mesenteric resistance arteries and aorta. Both amlodipine and felodipine caused a concentration-dependent relaxation of K^+-depolarized resistance arteries: with amlodipine 3×10^{-8} M and felodipine 10^{-9} M, complete relaxation was reached after 40 min and 10 min, respectively. Furthermore, in resistance arteries, the time course of action of both drugs was shortest in vessels with the smallest diameter. In aorta, both drugs caused a marked relaxation of K^+-induced tone, without reaching a maximal effect within 2 h. Recovery of K^+-induced tone after both drugs was complete in resistance arteries, but not aorta, within 2 h. In resistance arteries exposed to K^+ depolarization or noradrenaline, both drugs displayed the characteristics of 1,4-dihydropyridine Ca^{2+} channel antagonists. The results show that amlodipine was slower to have an effect than felodipine, but that both drugs acted fastest in the smallest arteries.

Keywords: Amlodipine; Felodipine; Smooth muscle, vascular; Drug effect; (Rat)

1. Introduction

Amlodipine is a newly developed 1,4-dihydropyridine Ca^{2+} channel antagonist (Burges et al., 1987). In clinical studies, amlodipine displays a markedly slower onset of action and longer time course of effect compared to earlier Ca^{2+} channel antagonists of the 1,4-dihydropyridine group (Opie, 1988). The slow onset and long duration of action of amlodipine is ascribed to a slow rate of absorption and distribution, low hepatic metabolism and slow kinetics at the dihydropyridine receptor. The latter is supported by most in vitro mechanical and radioligand binding studies (Garcha et al., 1993; Burges et al., 1987; Matlib et al., 1988; Nayler and Gu, 1991), studies which have predominantly been performed on large arteries. In the human coronary bed, however, the action of amlodipine is suggested to have a faster onset in smaller and distal conduit arteries compared to larger and more proximal conduit arteries (Godfraind et al., 1989). A faster onset in smaller arteries is also suggested from human in vivo studies, where the vascular resistance index and systemic arterial blood pressure are significantly lowered 10–15 min after i.v. injection of amlodipine (Silke et al., 1990). Thus, the time course of onset of amlodipine may be dependent on the size of the arteries examined, being faster in the smaller arteries. This conclusion is, however, made on the basis of comparisons of vessels from different species, and using different protocols. We have therefore found it important to investigate the effects of amlodipine on tone in arteries of different size in a single species, and using the same protocols. The work has been done using rat resistance arteries and aorta, and the actions of amlodipine have been compared with those of the previously well-investigated drug felodipine.

2. Materials and methods

2.1. Preparation

12- to 16-week-old male Wistar rats were killed with CO_2 on the day of the experiment. Segments (about 2 mm long) of resistance arteries (internal diameter ca. 200 μm) from the mesenteric bed or thoracic aorta were dissected free from surrounding tissue and

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mounted in a myograph allowing isometric wall tension measurement (Mulvany and Halpern, 1977). The artery segments were threaded onto two stainless-steel wires (diameter 40 μm), which were fastened to a force transducer and a micrometer, respectively, and placed in a 10-ml organ bath. After a rest period of 30–60 min, the arteries were set to a normalized internal diameter (l_1), estimated to be 0.9 times the internal diameter the arteries would have had in situ when relaxed and under a transmural pressure of 100 mm Hg (Mulvany and Halpern, 1977). The normalized internal diameter is where force development in arteries has been found to be maximal. After normalization, the vessels were activated in turn with 10 μM noradrenaline, high K^+ physiological salt solution (K^+-PSS) and 10 μM noradrenaline in K^+-PSS. Any vessel not contracting with a response corresponding to contraction against a pressure of 100 mm Hg (Mulvany and Halpern, 1977) was discarded. All artery segments whose tone was to be induced with K^+ depolarization were incubated in 1 μM phenoxybenzamine for 10 min and then washed for 30 min, in order to block α-adrenoceptors, to avoid the effects of neuronally released noradrenaline.

2.2. Protocol

Time-course experiments

The time course of the effects of amlodipine and felodipine was studied on contractions induced by depolarizing the arteries with K^+-PSS. Force was measured after 5 min, and this value was used as control for the later recovery responses. Forty minutes after induction of contraction, a single concentration of either amlodipine or felodipine was added to the organ bath, and the effect of the drug was studied for 120 min. The recovery of the K^+-induced responses was studied for another 120 min in the absence of Ca^{2+} channel antagonist. This was done by activating the artery segments with K^+-PSS for 5 min every 20 min. After all contractions, the organ bath (10 ml) was washed out 3 times. In the experiments with aorta, segments of resistance artery and aorta were mounted in the same myograph, in order to compare the time course of drug action in the two different arteries under exactly the same pharmacological conditions. In order to investigate if artery diameter affected the time course of action of amlodipine 3×10^{-8} M and felodipine 10^{-9} M, time-course experiments were also done in mesenteric resistance arteries larger (normalized internal diameter from about 250 to 400 μm) than the resistance arteries used in the other experiments.

Ca^{2+} concentration-response experiments

The effects of amlodipine and felodipine were investigated on cumulative Ca^{2+} concentration-response curves, using either K^+-PSS or 10 μM noradrenaline to induce tone. The artery segments were incubated with different concentrations of amlodipine or felodipine in Ca^{2+}-free K^+-PSS for 120 min, and the experiment was done in the continuing presence of the Ca^{2+} channel antagonist. Then, in order to deplete intracellular Ca^{2+} stores, the vessels were challenged with 25 mM caffeine in Ca^{2+}-free PSS for 30 s, followed by two 2-min washes in Ca^{2+}-free PSS. Subsequently, the vessels were exposed to increasing concentrations of $CaCl_2$, allowing 2 min equilibration without agonist in each new concentration of Ca^{2+} before inducing tone. Results are given relative to a control response in the same vessel, obtained before the incubation with the Ca^{2+} channel antagonist.

2.3. Solutions and drugs

The arteries were dissected, mounted and held relaxed in a physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 25, $CaCl_2$ 2.5, ethylenediaminetetraacetic acid (EDTA) 0.026, glucose 5.5. K^+-PSS was PSS in which NaCl was exchanged for KCl on an equimolar basis. Ca^{2+}-free PSS and Ca^{2+}-free K^+-PSS were standard solutions without $CaCl_2$. All solutions were held at 37°C and bubbled with 5% CO_2 in O_2 to give pH 7.4.

(−)-Noradrenaline hydrochloride (Sigma) and amlodipine besylate (Pfizer) were dissolved in distilled water. Stock solutions of phenoxybenzamine (Smith Kline and French) and felodipine (Astra) were made in ethanol and diluted in distilled water. Caffeine (Merck) was dissolved in Ca^{2+}-free PSS.

2.4. Statistics

Differences between means were tested for significance using a *t*-test. The correlation between normalized internal artery diameter and time course of action was examined using least-squares linear regression. For each concentration-response curve, an EC_{50} was estimated as the Ca^{2+} concentration causing a half-maximal response, using the commercially available GraphPAD program (GraphPad Software, San Diego, CA, USA). A pD_2 value was calculated as $-\log EC_{50}$. Values are given as means ± S.E.M. Comparison between groups was carried out by analysis of variance and subsequent *t*-test, correcting the *P*-value using the Bonferroni method. $P < 0.05$ was considered significant.

3. Results

3.1. Time-course experiments

Both amlodipine and felodipine caused a concentration-dependent relaxation of the rat mesenteric resis-

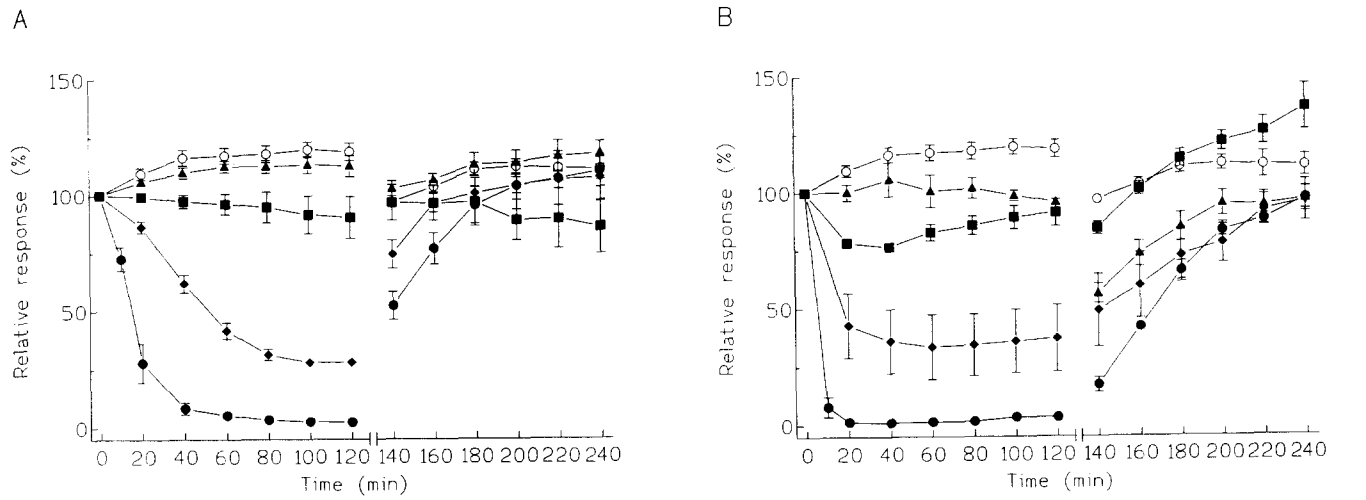

Fig. 1. Time course of relaxant action of (A) amlodipine and (B) felodipine in rat mesenteric resistance arteries precontracted by K^+ depolarization, and recovery of responses to the same stimuli after wash-out of the drugs. Gap in the abscissa indicates start of wash-out. Open circles are time-matched control responses. Concentrations of amlodipine in (A): (▲) 10^{-9} M, (■) 3×10^{-9} M, (◆) 10^{-8} M, (●) 3×10^{-8} M and of felodipine in (B): (▲) 3×10^{-11} M, (■) 10^{-10} M, (◆) 3×10^{-10} M, (●) 10^{-9} M. Points show means from 4 to 10 arteries, with limits of S.E.M. shown by vertical bars.

tance arteries precontracted by K^+ depolarization. In time-matched control experiments, no relaxation was observed. Fig. 1 shows the time course of the relaxant effect. The effect of amlodipine was characterized by a slow onset, amlodipine 10^{-8} M and 3×10^{-8} M reaching a maximal effect after 80 and 40 min, respectively, compared to felodipine 3×10^{-10} M and 10^{-9} M, where the maximal effect was seen after 40 and 10 min, respectively. Fig. 1 also shows that at the highest concentrations used, amlodipine (3×10^{-8} M) and felodipine (10^{-9} M) both caused almost complete relaxation. Amlodipine 10^{-8} M and 3×10^{-8} M caused $72 \pm 2\%$ ($n = 4$) and $98 \pm 1\%$ ($n = 7$) relaxation, respectively, compared to the effect of felodipine, where 3×10^{-10} M and 10^{-9} M caused $63 \pm 14\%$ ($n = 6$) and $97 \pm 1\%$ ($n = 4$) relaxation, respectively. The recovery of K^+-induced tone was slow for both drugs; however, recovery was complete within 2 h compared to the time-matched controls (Fig. 1). The time course of action of amlodipine 3×10^{-8} M and felodipine 10^{-9} M in rat mesenteric resistance arteries and aorta which were mounted pairwise in the same myograph, and thus exposed to the same conditions, is shown in Fig. 2. Compared to the resistance arteries the time course of action in

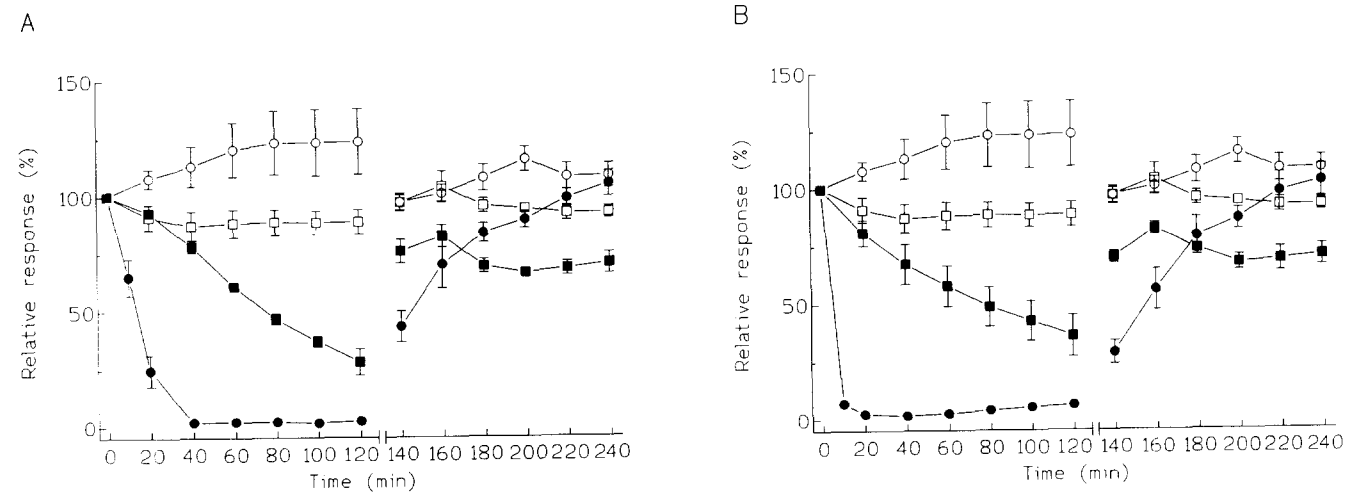

Fig. 2. Time course of relaxant action of (A) amlodipine 3×10^{-8} M and (B) felodipine 10^{-9} M (closed symbols) in rat mesenteric resistance arteries (circles) and rat aorta (squares) precontracted by K^+ depolarization, and recovery of responses to the same stimuli after wash-out of the drugs. Gap in the abscissa indicates start of wash-out. Open symbols are time-matched control responses. Points show means from 4 to 5 arteries, with limits of S.E.M. shown by vertical bars.

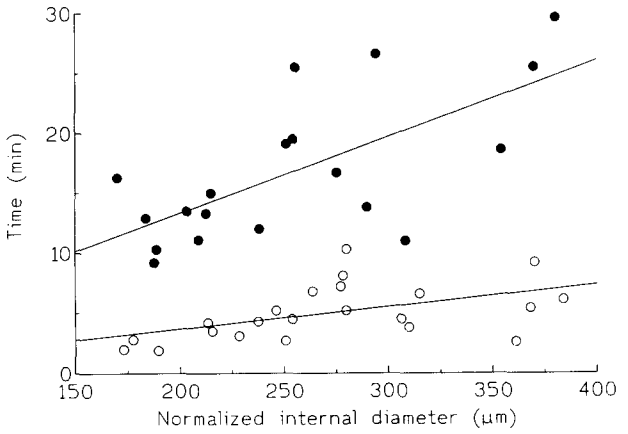

Fig. 3. Time taken by amlodipine 3×10^{-8} M (●, $n = 19$) and felodipine 10^{-9} M (○, $n = 22$) to reduce vessel tone to 50% of initial tone was correlated to the normalized internal diameter. The correlation coefficients are 0.67 and 0.49 for amlodipine and felodipine, respectively. Both slopes are significantly different from 0, $P = 0.0016$ (amlodipine) and 0.019 (felodipine).

aorta was much slower, amlodipine and felodipine causing $72 \pm 6\%$ ($n = 4$) and $64 \pm 9\%$ ($n = 4$) relaxation after 2 h, respectively, without the response having stabilized. Furthermore, in the aorta segments, the recovery of K^+-induced tone after 2 h of washout of Ca^{2+} channel antagonists was incomplete.

Pooling all mesenteric resistance arteries exposed to amlodipine 3×10^{-8} M in time course experiments ($n = 19$), the time for amlodipine to reduce vessel tone to 50% of the initial tone was significantly correlated to the normalized internal artery diameter. The slope (0.057 ± 0.015 min/μm) was significantly different from zero (Fig. 3). A similar significant correlation (slope 0.018 ± 0.006 min/μm) was found for vessels ($n = 22$) exposed to felodipine 10^{-9} M (Fig. 3). The latter slope was significantly less steep than that found for the amlodipine data.

3.2. Ca^{2+} concentration-response experiments

Both amlodipine and felodipine reduced the sensitivity to Ca^{2+} in a non-competitive concentration-dependent manner, as well as the maximal contraction in mesenteric arteries depolarized with K^+-PSS (Table 1). Also in the noradrenaline-activated vessels, amlodipine and felodipine progressively shifted the Ca^{2+} concentration-response curves to the right and caused a decrease of the maximal tissue response (Table 2). The effect of felodipine on the maximal responses was more pronounced than for amlodipine. The highest concentrations of felodipine (10^{-9} M) inhibited the maximal noradrenaline-stimulated responses to Ca^{2+} significantly more ($P < 0.01$) than did amlodipine (3×10^{-8} M). Both drugs were more potent in inhibiting responses to Ca^{2+} induced by K^+ depolarization compared to responses induced with noradrenaline; however, amlodipine was more selective than felodipine.

Table 1
Effect of amlodipine and felodipine on sensitivity and maximal response to Ca^{2+} in 125 mM K^+-stimulated rat mesenteric resistance arteries

	pD_2 (M)	Maximal response (%)
Control	3.42 ± 0.04	120.9 ± 5.5 (4)
Amlodipine		
1 nM	3.24 ± 0.05	86.3 ± 8.4 (4)
3 nM	3.17 ± 0.03	79.8 ± 10.1 [a] (6)
10 nM	3.08 ± 0.07 [a]	39.7 ± 5.6 [b] (4)
30 nM	3.05 ± 0.07 [b]	17.2 ± 3.1 [c] (6)
Felodipine		
0.03 nM	3.35 ± 0.02	94.0 ± 9.7 (6)
0.1 nM	3.23 ± 0.06	60.8 ± 8.8 [b] (6)
0.3 nM	3.06 ± 0.13 [a]	27.6 ± 3.9 [b] (4)
1 nM	ND	13.6 ± 4.5 [c] (4)

Values are means ± S.E.M. (number of vessels). Sensitivities are expressed as pD_2 values (M) and maximal response is percentage of control in the same artery. ND is not determined.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; compared to control.

4. Discussion

The major finding of the present study is that the time course of action of amlodipine and felodipine is dependent on both the type and the size of the artery examined. Thus, the time course was faster for both drugs in resistance arteries compared to aorta, and in resistance arteries the time course was faster the smaller the artery.

In mesenteric resistance arteries, vessels in which contraction has been shown to be crucially dependent

Table 2
Effect of amlodipine and felodipine on sensitivity and maximal response to Ca^{2+} in 10 μM noradrenaline-stimulated rat mesenteric resistance arteries

	pD_2 (M)	Maximal response (%)
Control	3.95 ± 0.06	118.2 ± 2.3 (4)
Amlodipine		
1 nM	3.59 ± 0.03 [c]	92.2 ± 7.0 [a] (8)
3 nM	3.46 ± 0.04 [b]	106.2 ± 3.7 (4)
10 nM	3.43 ± 0.09 [b]	103.4 ± 4.3 (4)
30 nM	3.22 ± 0.01 [c]	86.5 ± 4.0 (4)
Felodipine		
0.03 nM	3.59 ± 0.05	101.7 ± 0.7 (4)
0.1 nM	3.47 ± 0.09	91.0 ± 8.7 (6)
0.3 nM	3.33 ± 0.05 [a]	67.7 ± 7.9 [a] (6)
1 nM	3.34 ± 0.16 [a]	47.4 ± 10.9 [b] (7)

Values are means ± S.E.M. (number of vessels). Sensitivities are expressed as pD_2 values (M) and maximal response is percentage of control in the same artery.
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; compared to control.

on the influx of Ca^{2+} from the extracellular media (Cauvin et al., 1984), both amlodipine and felodipine (3×10^{-8} M and 10^{-9} M respectively) caused a nearly complete relaxation of K^+-induced contractions. The time course of this effect was different for the two drugs in that amlodipine was slower to have an effect compared to felodipine. However, we found a tendency towards a faster time course for amlodipine in resistance arteries compared to the equilibration time of about 3.5 h (Burges et al., 1987) and 2.5 h (Matlib et al., 1988) in previous mechanical experiments in rat aorta and 3.5 h in proximal human coronary arteries (Godfraind et al., 1989). This apparent discrepancy has never been reported nor examined in detail before. The difference could be due to differences in test protocols and drug concentrations used, but could also be due to a selectivity of amlodipine for smaller arteries (Godfraind et al., 1989).

4.1. Is the time course of drug action dependent on artery size?

The time course of action for both drugs was substantially slower in aorta segments compared to resistance arteries exposed to the same drug concentrations and experimental conditions. In the resistance arteries, both drugs caused complete relaxation, but in aorta, neither of the drugs reached its full effect within 2 h. Furthermore, unlike the resistance arteries, the responses to K^+-PSS did not recover completely during the 2-h wash-out period. The slow relaxation observed in the K^+-PSS contracted aorta segments is unlikely to be due to the structural characteristics of the aorta vascular wall. This is evidenced by the more than 90% relaxation seen 5 min after wash-out of K^+-PSS in the control aorta segments. However, the rate of relaxation after wash-out of K^+-PSS is dependent on the phenoxybenzamine treatment given to all the arteries, since relaxation of the aorta segments before the phenoxybenzamine treatment was noticeably slower. The results are in agreement with findings for amlodipine in rat aorta (Burges et al., 1987; Matlib et al., 1988) and felodipine in rat aorta (Kuroda et al., 1991) and rat tail artery (Hermsmeyer and Rusch, 1987). Thus, both drugs are able to relax both artery types, as can other dihydropyridine Ca^{2+} channel antagonists (Wadsworth, 1993).

The importance of artery size for the time course of action is further supported by the significant correlation in resistance arteries between the time course of relaxation and artery internal diameter. For both drugs we have found that the smaller the artery the faster the effect, although for any given vessel diameter amlodipine was slower to have an effect than felodipine. Since the in vivo target of the drugs is resistance arteries, this may suggest that the slow clinical onset of amlodipine action when administered orally is mostly determined by its slow absorption and distribution in the body. The correlation for felodipine data was less strong and the slope significantly less steep than that for the amlodipine data. This could suggest that, in addition to the importance of artery type and size for the time course, also differences between amlodipine and felodipine determine the time course.

4.2. Possible mechanism for the slower action of amlodipine

Part of the reason for the slower action of amlodipine in the aorta compared to the resistance vessels may be related to the difference in calcium metabolism in different sections of the vasculature (Bevan et al., 1986). However, it is likely that kinetics at the receptor site play an important role. Amlodipine is a weak base with a pK_a of 8.6 and under physiological conditions (pH 7.4) amlodipine is about 94% ionized. This is believed to be the molecular basis for the slower onset of action of amlodipine. Compared to this, felodipine has a pK_a of less than 1, like the dihydropyridine prototype nifedipine, and is almost completely non-ionized. The importance of charge for the time course of action is emphasized in a series of 1,4-dihydropyridines (Kwon et al., 1990), where the inhibitory effect against K^+-induced tension in rat tail artery was rapid in onset and offset in neutral analogues, but slow in analogues bearing a positively charged side chain. The importance of charge is also supported by patch-clamp experiments in isolated guinea pig ventricle cells. The rates of development and recovery of Ca^{2+} channel block was found to be faster and to resemble the block caused by neutral dihydropyridines under alkaline conditions that favour neutral amlodipine molecules (Kass and Arena, 1989).

Compared to the time course in mechanical experiments, amlodipine has been found to inhibit Ca^{2+} channel currents with a relatively rapid onset of action in single smooth muscle cells isolated from rabbit ear artery (Hughes and Wijetunge, 1993). It was therefore suggested that the slow onset in intact arteries reflected difficulty for the charged amlodipine molecule to gain access to multicellular preparations (Hughes and Wijetunge, 1993). Diffusion of amlodipine into larger arteries would be slowed because of the limited ability to cross cell membranes (Kass et al., 1991), and thus be part of the mechanism causing the slow onset of action. Such a scheme is supported by our finding in resistance arteries, where the time course of amlodipine action was strongly dependent on vessel size. Also, the uncharged felodipine molecule would diffuse faster into the tissue, this being reflected in the slope of the regression line for felodipine being less than one-third of that found for amlodipine. In aorta this diffusion

effect would be masked by the basic mechanism for mobilization of Ca^{2+}, minimizing the difference in time course between amlodipine and felodipine in this particular type of artery.

The faster initial recovery of K^+-PSS-induced tone in aorta segments compared to resistance arteries was a rather unexpected finding. However, this finding could also to some extent be explained by the diffusion of the Ca^{2+} channel antagonists. In the aorta segments, the drugs reach the innermost cells in a lower concentration than in the innermost cells of the resistance arteries, which are bathed by the full concentration, as evidenced by the complete relaxation. Thus, all smooth muscle cells in the resistance arteries will be equally affected by the drugs, and the recovery will reflect the wash-out of the drug. In the aorta segments, the innermost cells will regain their contractile effect relatively fast, and in this way cause the rapid initial recovery.

4.3. Effects of amlodipine and felodipine on Ca^{2+} contractions

Dihydropyridine Ca^{2+} channel antagonists are thought to exert their effect mainly by inhibiting the influx of Ca^{2+} through potential-sensitive channels, and only cause minor inhibition of influx through other membrane Ca^{2+} channels. This selectivity has been proposed to cause the characteristic selectivity of dihydropyridine Ca^{2+} channel antagonists for depolarizing stimuli compared to catecholamine-induced contractions (Struyker Boudier et al., 1990). A selectivity for depolarizing stimuli over agonist-induced contractions has been found for amlodipine in previous experiments in rat aorta (Matlib et al., 1988) although this finding is not uniform (Burges et al., 1987). Furthermore, in human subcutaneous resistance arteries, amlodipine has also been found to cause a marked relaxation of responses to noradrenaline, although the concentration of amlodipine used was rather high (1 μM) (Garcha et al., 1991). The ability of felodipine to relax tone induced by K^+ depolarization more than tone induced by noradrenaline has previously been reported in rat mesenteric resistance arteries (Nyborg and Mulvany, 1984). In the present study, the selectivity was found for both drugs, amlodipine and felodipine inhibiting responses induced by K^+ depolarization more than responses induced by noradrenaline. This discrimination was particularly true for amlodipine, although it is possible that the apparent ineffectiveness of amlodipine against noradrenaline induced responses is related to its slower onset of action.

In conclusion, we have found the time course of action of amlodipine and felodipine to be dependent on the type and size of the artery examined. The time course of the inhibitory action of both drugs was slower in aorta segments compared to resistance arteries, and in resistance arteries the onset was relatively faster in the smallest vessels. This suggests that the slow onset of amlodipine observed in vivo may be mostly determined by the pharmacokinetic profile of the drug.

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