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Vascular reactivity to nifedipine and Ca^{2+} in vitro: the role of preactivation, wall tension and geometry

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Abstract

The purpose of the study was to investigate the influence of preactivation, wall tension and geometry on the reactivity of porcine coronary arteries to nifedipine and extracellular Ca^{2+} in vitro. Porcine large coronary arteries were mounted as ring and cylindrical preparations and studied by wire- and balloon-based techniques. The sensitivity and maximal responses to nifedipine were more pronounced in 25 mM K^+ compared to 10 μM prostaglandin $F_{2\alpha}$-contracted preparations. Vascular sensitivity to nifedipine and Ca^{2+} was enhanced under isometric compared to isobaric conditions. Under isometric conditions in the presence of 25 mM K^+, coronary rings were more sensitive to nifedipine, but less sensitive to Ca^{2+} compared to cylindrical segments. In cylindrical segments, circumferential and axial tension increases augmented the extracellular Ca^{2+}-dependent spontaneous resting tone and the sensitivity to extracellular Ca^{2+}. Coronary rings showed no resting tone at various resting tensions. These results suggest that preactivation, wall tension and vessel geometry are important determinants of Ca^{2+}-influxes via nifedipine-sensitive voltage-gated Ca^{2+} channels. Furthermore, axial wall tension appears to be a modulator of nifedipine-insensitive transmembrane Ca^{2+}-influx that may play a role for the tone and reactivity in large coronary arteries.

Keywords: Circumferential stretch; Axial stretch; Impedance planimetry; Coronary artery, large; (Pig); Tone; Ca^{2+}-channel, voltage-gated

1. Introduction

The fact that vasoconstriction of large coronary arteries may cause angina pectoris and contribute to myocardial infarct and sudden death, draws attention to the mechanisms involved in regulation of the tone and reactivity in these vessels (Hellstrom, 1979; Velican, 1980). Previous studies have indicated that neural, humoral as well as mechanical factors are important determinants of vascular tone and may play a role in the coronary flow-regulation (Bassenge and Heusch, 1990; Osol, 1995). The role of mechanical factors in large coronary arteries is emphasized by the large circumferential and axial deformations these vessels are exposed to in vivo (Osol, 1995).

Johnson (1980) proposed that wall tension is an important regulator of the tone and vascular reactivity. When a vessel is contracted, wall tension changes are opposed under isobaric and isometric conditions. Thus, it decreases under isobaric and increases under isometric conditions. The changing wall tension accompanying the contraction will have a feedback effect on the actual vascular reactivity. This feedback effect is negative under isobaric and positive under isometric conditions. Therefore, techniques allowing studies in isolated blood vessels under both isobaric and isometric conditions may give insights into the role of wall tension in the regulation of the vascular reactivity.

In a previous study using a bimodal balloon-based technique, we have demonstrated that at the same initial load the vascular sensitivity of cylindrical segments from porcine large coronary arteries to K^+ and various agonists was enhanced under isometric compared to isobaric conditions (Tankó et al., 1998b). These findings are in agree-

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ment with results on small mesenteric arteries (Van Bavel and Mulvany, 1994). Moreover, Buus et al. (1994) reported that the presence of D600 (methoxy-verapamil), a voltage-gated Ca^{2+} channel blocker, caused a 50% inhibition of maximal contractile responses to noradrenaline in vessel rings under isometric conditions. No inhibition, however, was seen in cannulated segments under isobaric conditions. A similar low efficiency of nifedipine was observed in an in vivo setup (Liu et al., 1994). These observations suggest that differences in vascular reactivity under isobaric and isometric conditions could be due to differences in Ca^{2+}-influx via L-type voltage-gated Ca^{2+} channels.

Studies on cannulated segments from various vascular beds have demonstrated that increasing resting tension induces membrane depolarization with concomitant increases in transmembrane Ca^{2+}-influxes via voltage-gated Ca^{2+} channels (Schubert and Mulvany, 1999). In contrast, change in resting tension was not associated with alterations in the resting membrane potential in wire-mounted rings (Harder et al., 1983; Halpern and Kelley, 1991). Moreover, the vascular sensitivity of wire-mounted rings to Ca^{2+} does not change with increasing resting wall tension (Price et al., 1981; Herlihy and Berardo, 1986). Thus, geometry of the preparation may have important implications for membrane potential and thereby the Ca^{2+} influx via L-type voltage-gated Ca^{2+} channels.

The purpose of the present study was to investigate how preactivation, wall tension and geometry of the preparation influence the nifedipine-sensitive transmembrane Ca^{2+} influx in porcine coronary arteries in vitro. This was addressed by the following experimental approaches: (1) The influence of preactivation was studied by comparing the action of nifedipine in preparations activated either directly by 25 mM K^+ or via receptors by prostaglandin $F_{2\alpha}$. (2) The influence of wall tension was addressed by comparing the concentration–response relationship for nifedipine and Ca^{2+} under isobaric and isometric conditions. (3) The influence of geometry was studied by comparing the action of nifedipine and Ca^{2+} in cylindrical and ring preparations. In addition, it was studied whether changes in resting tension in cylindrical (circumferential and axial) and in ring (circumferential) preparations influence the transmembrane Ca^{2+}-influx under resting and K^+-depolarized conditions.

2. Materials and methods

Hearts from Danish Landrace–Yorkshire pigs (70–90 kg) were obtained at a local abattoir. The left anterior descending coronary artery was dissected and trimmed of the adherent fat and connective tissue under a stereo microscope while submerged in ice-cold (4°C) physiologic saline solution (PSS). To reduce intertissue variation, two 30 mm long cylindrical segments and a 3-mm long ring cut from the same coronary artery were used for balloon-based and wire-based investigations, respectively.

In a previous study, we have demonstrated that, providing careful matching of vessel and probe size and carrying out the probe insertion with precaution, endothelial cell function can be preserved in porcine coronary artery segments mounted in the normal vessel configuration (Tankó et al., 1999). However, the presence or absence of endothelium cannot be verified functionally by adventitially administered endothelium-dependent relaxant agents due to presence of an effective diffusion barrier in the vessel wall for these substances (Tankó et al., 1999). Consequently, evaluation of the role of endothelium was carried out on everted (endothelial cell layer outside the vessel lumen) preparations with and without endothelium. The endothelial cell layer was removed by gently rubbing on the surface of the everted preparation using moistened cotton wool. The presence or absence of endothelial cell layer was evaluated by inducing a contraction with prostaglandin $F_{2\alpha}$ and then adding 0.3 μM bradykinin to the organ bath. Relaxation greater than 50% was taken as evidence of endothelial integrity, while total inhibition of the relaxation to bradykinin was indicative of successful mechanical removal of the endothelial cells.

2.1. Impedance planimetry

The arterial segments were mounted on the cannulas of vessel holders and fastened with 4–0 ligatures. Experiments took place in 50 ml organ baths filled with PSS and continuously bubbled with 5% CO_2 in 95% O_2 at 37°C to give a pH of 7.4. After a 60-min equilibration period, the impedance planimetry probes were gently inserted into the vessel lumen. The probe applied in the present study has earlier been described (Tankó et al.,1998b). In brief, it consists of a four-electrode impedance measuring system located inside a ~25-mm long, thin-walled (50 μm), non-conducting polyurethane balloon (diameter 6 mm) mounted on a 70-mm long 3 F probe. The vessel segment was axisymmetrically loaded by distending the cylindrical balloon with electrically conducting fluid (0.09% NaCl) from a level container through an infusion channel (diameter ~0.5 mm) of the probe. Previous investigations showed that resting tensions corresponding to a luminal pressure of 60 mm Hg ensure optimal contractile responses in porcine coronary artery segments under both isobaric and isometric conditions (Tankó et al., 1998b). Therefore, all investigations with the impedance planimetry technique, if not otherwise indicated, were carried out at 60 mm Hg.

The bimodal (isobaric and isometric) applicability of the impedance planimetry technique lies in its ability to measure luminal cross-sectional area and pressure simultaneously. When the vessel is subjected to a constant luminal pressure and vascular responses are measured as changes in luminal cross-sectional area, the system mode is isobaric. Whereas, if the cross-sectional area of the balloon is

kept constant, by closing the outlet from the balloon by a stop cock, and vascular responses are measured as changes in luminal pressure, the system mode is isometric.

The balloon and thereby vessel cross-sectional area was estimated by measuring the electrical impedance of the saline inside the balloon using two electrodes for excitation and two interposed electrodes for detection as earlier described (Harris et al., 1971; Gregersen et al., 1988; Tankó et al., 1998b). Pressure inside the balloon and thereby in the vessel lumen was estimated by a low compliance external pressure transducer (Uniflow™, Baxter, USA) coupled to the infusion channel of the probe. Pressure transducers were set at the fluid level of the organ bath.

2.2. Wire-based isometric tension technique

The arterial rings were suspended vertically in a 30-ml organ bath on two stainless steel hooks. The organ bath was filled with PSS and continuously bubbled with 5% CO_2 in 95% O_2 at 37°C to give a pH of 7.4. The upper hook was connected to a manipulator and a force transducer (model Ft 0.3, Grass Instrument). After 60 min of equilibration, the ring was progressively stretched by the manipulator over a period of 30–40 min to 20 mN where contractile responses to 125 mM K^+ are optimal (Tankó et al., 1998a). Vascular responses were measured as changes in isometric force and recorded on a R611 Beckman polygraph.

2.3. Experimental protocols

2.3.1. Vascular sensitivity to nifedipine

The effect of cumulatively (0.1 nM–1 μM) administered nifedipine was studied in coronary artery preparations contracted either with 10 μM prostaglandin $F_{2\alpha}$ or 25 mM K^+. It is difficult to wash out nifedipine from the arterial wall during the experimental period (Mikkelsen and Lederballe Pedersen, 1982). Therefore, the influence of recording mode was evaluated in segments from the same coronary artery (one segment for isobaric and one for isometric testing). In parallel, a ring preparation, cut from in between the segments used for balloon-based studies, was studied by the wire-based isometric technique.

After testing for viability and obtaining a reproducible contractile response to 125 mM K^+ under isometric conditions, the vessel was contracted either with 10 μM prostaglandin $F_{2\alpha}$ or 25 mM K^+. When the contractile response reached steady state, nifedipine was added cumulatively into the organ bath awaiting steady state responses. Stability of contractile responses was evaluated in parallel time control experiments.

The role of endothelium in the determination of vascular sensitivity to cumulatively (0.1 nM–1 μM) administered nifedipine was assessed in 10 μM prostaglandin $F_{2\alpha}$-contracted everted coronary preparations with and without endothelium according to the above described protocol.

2.3.2. Vascular sensitivity to extracellular Ca^{2+} in the presence of 25 mM K^+

The vascular sensitivity to cumulatively administered (0.05–4 mM) Ca^{2+} was studied in 25 mM K^+ activated preparations. To remove Ca^{2+} from the organ bath, the preparations were repeatedly rinsed with 0.1 and 0.01 mM EGTA containing Ca^{2+}-free PSS. Then, the segments were exposed to 0.01 mM EGTA-containing Ca^{2+}-free K^+-PSS (25 mM K^+) and Ca^{2+} was administered cumulatively awaiting steady state responses at each concentration.

2.3.3. Effect of extracellular Ca^{2+} removal and 0.3 μm nifedipine on resting tone

After testing for viability, the rings and the cylindrical segments were allowed to equilibrate in 1.5 mM Ca^{2+} containing PSS for 30–40 min at resting conditions corresponding to 20 mN and 60 mm Hg, respectively. Then, Ca^{2+} was removed by repeatedly changing the solution in the organ bath with 0.1 mM EGTA-containing Ca^{2+}-free PSS. When the vascular response reached steady state, the tone was restored by the readdition of Ca^{2+}. Subsequently, the effect of 0.3 μM nifedipine was evaluated.

To investigate whether the magnitude of the extracellular Ca^{2+}-dependent spontaneous resting tone is influenced by resting circumferential wall tension, the protocol was carried out in groups of cylindrical coronary segments subjected to 20, 60 or 100 mm Hg and in groups of wire-mounted rings subjected to 10, 20, or 30 mN.

To address the role of axial tension, the extracellular Ca^{2+}-dependent resting tone was studied under isometric conditions at 60 mm Hg in cylindrical segments mounted either at non-stretched (L_0) or 1.2 L_0 axial length. Pilot studies indicated that midwall strain shows no significant differences at L_0 or 1.2 L_0 axial lengths when the cylindrical segments are distended by pressures equal or higher than 60 mm Hg.

2.3.4. Vascular sensitivity to extracellular Ca^{2+} at different resting tensions

Pilot studies indicated that repeated concentration–response curves for Ca^{2+} constructed in the presence of 125 mM K^+ exhibited significant rightward shifts and this desensitisation of the preparation could not be eliminated by several hours of equilibration before a second concentration–response curve. Therefore, only one concentration–response curve for Ca^{2+} was constructed in each preparation.

To evaluate the influence of circumferential resting tension on the vascular sensitivity of cylindrical coronary artery segments to extracellular Ca^{2+}, concentration–response curves for Ca^{2+} were constructed in three groups of preparations subjected to 20, 60, or 100 mm Hg. To investigate the role of axial tension, concentration–re-

sponse curves for Ca^{2+} were constructed at 60 mm Hg in two groups of cylindrical segments mounted either at L_0 or 1.2 L_0 axial length. Both types of investigations were carried out under isometric conditions in the presence of 125 mM K^+, similarly to the protocol described under Section 2.3.3.

2.4. Solutions and drugs

The composition of the PSS was (in mM) 100 NaCl, 15 $NaHCO_3$, 4.7 KCl, 10 $MgCl_2$, 1.2 $NaPO_4$, $2H_2O$, 1.5 $CaCl_2$ and 11.1 glucose and 0.027 EDTA. In experiments where K^+-PSS was used, NaCl was substituted by KCl on an equimolar basis. Solutions were prepared using analytical grade chemicals and twice distilled water.

Drugs and substances used in the study were: nifedipine (Bayer, Denmark), prostaglandin $F_{2\alpha}$ (Dinoprost, Upjohn, Germany) and bradykinin HCl (Sigma, St. Louis, USA). Nifedipine was protected from light, and all experiments with nifedipine were carried out in a darkened room (light intensity < 10 lx). Nifedipine was dissolved in 96% ethanol. The final vol.% of alcohol was under 0.01% and had no direct effect on active vessel tone.

2.5. Data analysis

The concentration–response curves were expressed as percentage of the respective maximal contractile response and computer-fitted using the Graphpad Prism 2.0 software (Institute for Scientific Information, CA, USA). The concentration–response curves were fitted to the classical Hill equation: $R/R_{max} = A(M)^n(A(M)^n + EC_{50}(M)^n)^{-1}$, where R/R_{max} is the relative response to the effective concentration of the substance, $A(M)$, and $EC_{50}(M)$ is the concentration of the substance required to give half-maximal response (R_{max}) when $A(M)$ and $EC_{50}(M)$ are given in molar concentration. n is the curve fitting parameter or Hill coefficient. In the nifedipine experiments, maximal responses (R_{max}) refer to responses evoked by the highest concentration applied of the drug.

The results are expressed as means ± S.E.M, and the concentration–response curves presented on a semi-logarithmic scale. Differences between pD_2 values were analysed by unpaired or paired *t*-test. If more than two groups of data were compared, the analysis was performed by one-way analysis of variance (ANOVA) followed by Newman–Keuls' multiple comparison test. Data from the investigations addressing resting tone were analysed by two-way ANOVA. Probability levels under 5% were considered as significant.

3. Results

3.1. Vascular sensitivity to nifedipine

Prostaglandin $F_{2\alpha}$ (10 μM) and 25 mM K^+-induced contractions of comparable amplitude in the porcine coronary artery preparations. These contractile responses were stable during the experimental periods. Under isometric conditions, contractile responses of ring and cylindrical preparations to 10 μM prostaglandin $F_{2\alpha}$ showed no significant differences. Thus, contractile responses expressed as percentage of the corresponding 125 mM K^+ responses in rings and cylindrical preparations were 52 ± 4% ($n = 5$) and 59 ± 6% ($n = 5$), respectively. In contrast, under isometric conditions, contractile responses to 25 mM K^+ were significantly larger in cylindrical segments 64 ± 3% ($n = 5$) compared to wire-mounted rings 45 ± 3% ($n = 5$, $p < 0.05$, paired *t*-test). Time-course of precontractile responses induced by 10 μM prostaglandin $F_{2\alpha}$ was similar under isobaric and isometric conditions, whereas 25 mM K^+-induced responses were markedly longer under isobaric compared to isometric conditions (Fig. 1).

Cumulatively administered (0.1 nM–1 μM) nifedipine caused concentration-dependent inhibition of contractile responses both to 25 mM K^+ and 10 μM prostaglandin $F_{2\alpha}$. The vascular sensitivity and the maximal inhibitory response to nifedipine were consistently enhanced in 25 mM K^+ as compared to 10 μM prostaglandin $F_{2\alpha}$-contracted preparations (Table 1).

The vascular sensitivity of cylindrical coronary segments to nifedipine was significantly enhanced under iso-

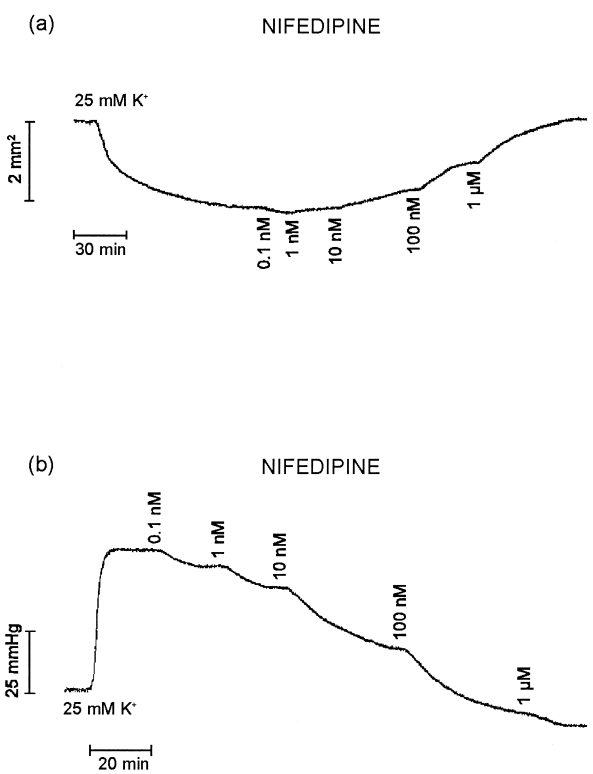

Fig. 1. Original trace recordings of the effect of cumulatively administered nifedipine (0.1 nM–1 μM) under isobaric (a) and isometric (b) conditions in 25 mM K^+-contracted segments from the same porcine coronary artery. Note the longer duration of the contractile response to 25 mM K^+ and completion of the concentration–response curve for nifedipine under isobaric compared to isometric conditions.

Table 1
pD_2 values and maximal inhibitory responses to the highest concentration (1 μM) of nifedipine applied either in 10 μM prostaglandin $F_{2\alpha}$ or 25 mM K^+-contracted preparations from the same porcine coronary artery under various experimental conditions
Data are expressed as means ± S.E.M. of five left anterior descending coronary arteries from different animals. $p < 0.05$, significantly different (one-way ANOVA followed by Newman–Keuls' test).

Nifedipine	Cylindrical segment				Ring	
	Isometric, prostaglandin $F_{2\alpha}$	Isometric, K^+	Isobaric, prostaglandin $F_{2\alpha}$	Isobaric, K^+	Isometric, prostaglandin $F_{2\alpha}$	Isometric, K^+
pD_2	7.54 ± 0.07^a	$8.34 \pm 0.14^{a,b}$	6.81 ± 0.08	7.63 ± 0.15^b	7.36 ± 0.10^c	$8.68 \pm 0.04^{b,d}$
% Inhibition	79 ± 2	$125 \pm 4^{b,d}$	73 ± 2	111 ± 5^b	75 ± 3	96 ± 2^b

[a] Versus isobaric cylindrical segment precontracted with the same agent (K^+ or prostaglandin $F_{2\alpha}$).
[b] Versus the same type of preparation (cylindrical segment or ring) precontracted under the same condition (isometric or isobaric) with prostaglandin $F_{2\alpha}$.
[c] Versus isobaric cylindrical segment precontracted with the same agent (K^+ or prostaglandin $F_{2\alpha}$).
[d] Versus isometric ring precontracted with the same agent (K^+ or prostaglandin $F_{2\alpha}$).

metric as compared to isobaric conditions both in 25 mM K^+ and 10 μM prostaglandin $F_{2\alpha}$-contracted preparations (Fig. 2a,b and Table 1). The vascular sensitivity to nifedipine in wire-mounted rings under isometric conditions was also enhanced compared to cylindrical segments studied under isobaric conditions (Fig. 2a,b and Table 1).

When comparing the two isometric preparations, 25 mM K^+-contracted wire-mounted rings were more sensitive to the inhibitory effect of nifedipine as compared to cylindrical segments (Fig. 2b, Table 1). Furthermore, the maximal responses to nifedipine also showed marked differences between cylindrical and the wire-mounted preparations (Fig. 2b). Nifedipine decreased the resting tone of cylindrical segments both under isometric and isobaric conditions, but not in the parallely investigated wire-mounted rings (Fig. 2b).

The role of endothelium in the vascular sensitivity to nifedipine under the different experimental conditions was evaluated in 10 μM prostaglandin $F_{2\alpha}$-contracted everted coronary segments with or without endothelium. Presence or absence of endothelial cell layer was indicated by the vascular response to 0.3 μM bradykinin (Table 2). Nifedipine induced comparable inhibitions of the prostaglandin $F_{2\alpha}$-induced contractile responses in endothelium-intact and endothelium-denuded preparations under both isobaric and isometric conditions, but in both type of preparations the vascular sensitivity to nifedipine was consistently enhanced under isometric compared to isobaric conditions (Table 2).

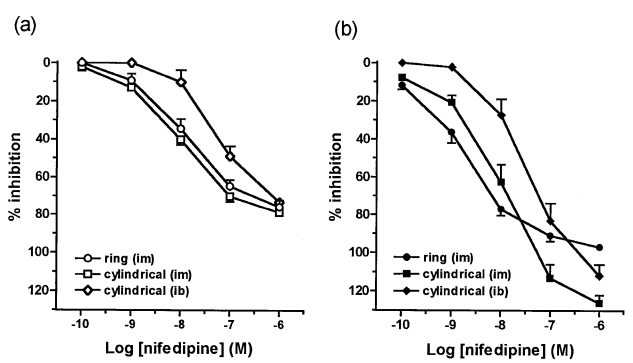

Fig. 2. (a) Concentration–response relationship for nifedipine in 10 μM prostaglandin $F_{2\alpha}$-contracted wire-stretched rings (im, isometric) and cylindrical segments (im, isometric; ib, isobaric) from the same coronary artery. (b) Concentration–response relationship for nifedipine in 25 mM K^+-activated wire-stretched rings (im, isometric) cylindrical segments (im, isometric; ib, isobaric) from the same coronary artery. Data are expressed as means ± S.E.M of five coronary arteries from different animals.

3.2. Vascular sensitivity to extracellular Ca^{2+} in the presence of 25 mM K^+

Cumulatively administered Ca^{2+} induced concentration-dependent contractions in the presence of 25 mM K^+ both under isobaric and isometric conditions (Fig. 3). The vascular sensitivity of cylindrical segments to Ca^{2+} was significantly enhanced under isometric compared to isobaric conditions (Fig. 3) with pD_2 values of 3.35 ± 0.06 and 2.92 ± 0.06 ($P < 0.05$, one-way ANOVA followed by Newman–Keuls' test, $n = 5$), respectively. Furthermore, under isometric conditions, the vascular sensitivity to Ca^{2+} was enhanced in cylindrical segments compared to wire-mounted rings with pD_2 values of 3.35 ± 0.06 and 3.15 ± 0.05 ($P < 0.05$, one-way ANOVA followed by Newman–Keuls' test, $n = 5$), respectively.

3.3. Effect of extracellular Ca^{2+} removal and 0.3 μM nifedipine on resting tone

Cylindrical segments studied at 60 mm Hg exhibited spontaneous resting tone that was abolished upon removal of extracellular Ca^{2+} and the addition of 0.3 μM nifedipine to the organ bath. In contrast, Ca^{2+} removal or nifedipine addition revealed no spontaneous resting tone in the wire-mounted rings stretched to 20 mN.

The spontaneous resting tone in cylindrical segments was related to the degree of resting tension in the vessel wall. At a resting tension corresponding to 20 mm Hg, no tone was present. The tone became considerable at 60 mm Hg with further increase at 100 mm Hg. In five experiments, resting tone in wire-mounted rings subjected to

Table 2
pD_2 values and maximal inhibitory responses to the highest concentration (1 μM) of nifedipine applied in 10 μM prostaglandin $F_{2\alpha}$-contracted everted coronary segments with and without endothelium
Data are expressed as means ± S.E.M. of five left anterior descending coronary arteries from different animals.

			Nifedipine (0.1 nM–1 μM)		Bradykinin (0.3 μM)
			pD_2	% Maximal inhibition	% Maximal relaxation
Cylindrical segment	isobaric	endothelium (+)	6.90 ± 0.12	67 ± 2	102 ± 4*,[a]
		endothelium (−)	6.96 ± 0.15	68 ± 3	1 ± 1
	isometric	endothelium (+)	7.70 ± 0.11*,[b]	73 ± 3	100 ± 5*,[a]
		endothelium (−)	7.78 ± 0.09*,[b]	75 ± 3	3 ± 1
Ring	isometric	endothelium (+)	7.53 ± 0.11*,[b]	70 ± 2	93 ± 4*,[a]
		endothelium (−)	7.60 ± 0.10*,[b]	71 ± 3	2 ± 1

* $p < 0.05$, significantly different.
[a] Versus corresponding endothelium (−) preparation (unpaired *t*-test).
[b] Versus corresponding isobaric value (one-way ANOVA followed by Newman–Keuls' test).

resting tensions of 10, 20 and 30 mN could not be revealed. Fig. 4 shows the effect of Ca^{2+} removal and 0.3 μM nifedipine on a porcine coronary artery when studied as a cylindrical segment at 100 mm Hg or as a wire-mounted ring at 30 mN. Fig. 5a shows the magnitude of the spontaneous tone in cylindrical segments at 20, 60 and 100 mm Hg. The corresponding vessel dimensions and resting tensions are indicated in Table 3.

The spontaneous resting tone at 60 mm Hg was significantly more prominent in cylindrical segments mounted at 1.2 L_0 compared to L_0 (Fig. 5b). Resting circumferential tension at 60 mm Hg in segments mounted at L_0 (16 ± 1 N m^{-1}; $n = 10$) and 1.2 L_0 (15 ± 1 N m^{-1}; $n = 10$) were not different.

3.4. Vascular sensitivity to extracellular Ca^{2+} at different resting tensions

Concentration–response curves for Ca^{2+} (0.05–4 mM) exhibited significant leftward shifts with increasing resting tension in the vessel wall (Fig. 6a, Table 2). The pD_2 value obtained at 60 mm Hg (3.95 ± 0.03, $n = 10$) was significantly higher compared to that obtained at 20 mm Hg (3.68 ± 0.03, $n = 10$, $p < 0.05$, one-way ANOVA followed by Newman–Keuls' test). The pD_2 value obtained at 100 mm Hg (4.18 ± 0.03, $n = 10$) was significantly higher than those obtained at 20 mm Hg (3.68 ± 0.03, $n = 10$) and 60 mm Hg (3.95 ± 0.03, $n = 10$), respectively ($p < 0.05$, one-way ANOVA followed by Newman–Keuls' test). Active tensions induced by cumulatively added Ca^{2+} were smaller at 20 mm Hg (22 ± 2 N m^{-1}, $n = 10$) compared to at 100 mm Hg (32 ± 3 N m^{-1}, $n = 10$,

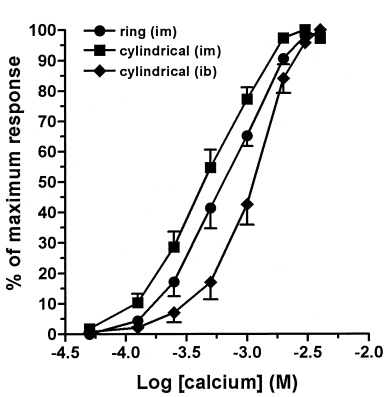

Fig. 3. Concentration–response relationship for Ca^{2+} in the presence of 25 mM K^+ studied in wire-mounted rings (im, isometric) and in cylindrical segments (im, isometric; ib, isobaric) from the same coronary artery. Data are expressed as means ± S.E.M of five coronary arteries from different animals.

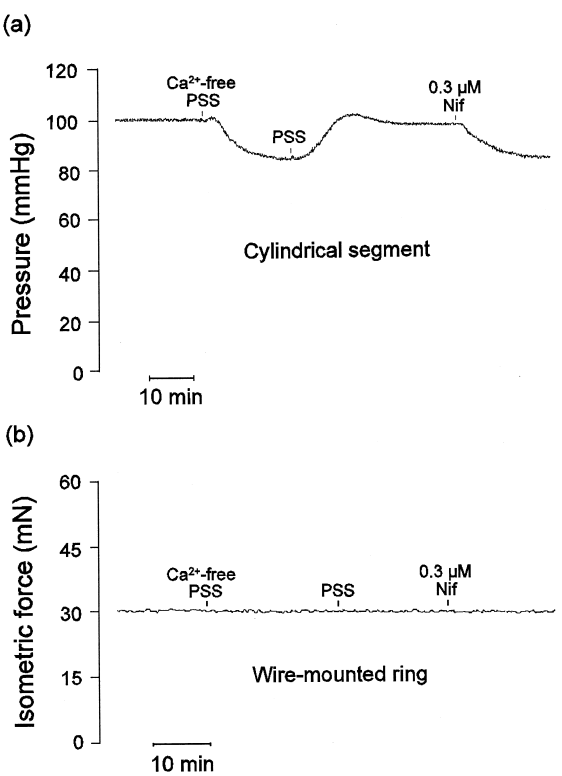

Fig. 4. Original trace recordings showing the effect of removal of extracellular Ca^{2+} and 0.3 μM nifedipine (Nif) on the resting tone of a porcine coronary artery when studied in a cylindrical segment (a) and a wire-mounted ring (b).

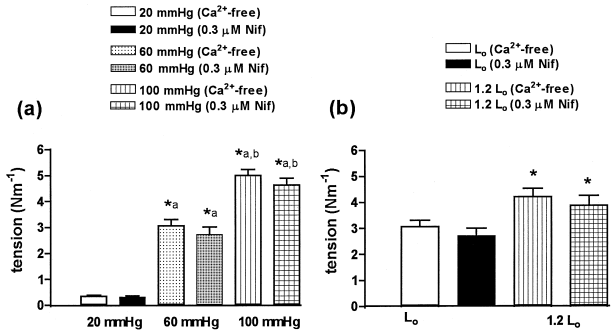

Fig. 5. (a) The effect of extracellular Ca^{2+} removal and 0.3 μM nifedipine (Nif) on the resting tone of porcine coronary arteries developed at 20, 60 or 100 mm Hg. Corresponding inner radii and resting tensions are indicated in Table 2. (b) The effect of extracellular Ca^{2+} removal or 0.3 μM nifedipine (Nif) on the resting tone at 60 mm Hg in porcine coronary arteries mounted either at L_0 or 1.2 L_0. Data are expressed as means ± S.E.M of eight to ten coronary arteries from different pigs. (* $p < 0.05$, two-way ANOVA). (a) Compared to corresponding type of experiments at 20 mm Hg, (b) compared to corresponding type of experiments at 60 mm Hg.

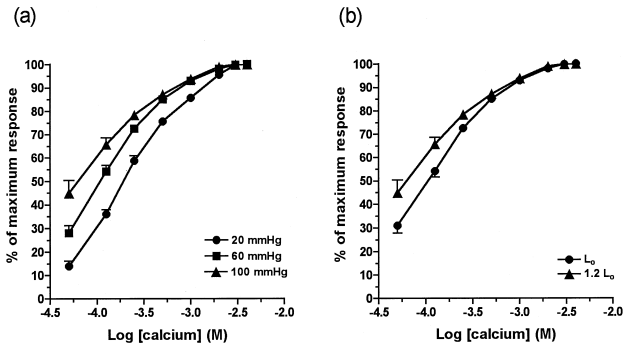

Fig. 6. (a) Vascular sensitivity to extracellular Ca^{2+} studied in cylindrical coronary segments mounted at in situ axial length (L_0) and subjected to 20 ($n = 10$), 60 ($n = 10$), or 100 mm Hg ($n = 10$). Concentration–response curves were constructed under isometric conditions in the presence of 125 mM K^+. Corresponding inner radii and resting tensions are indicated in Table 2. (b) Vascular sensitivity to extracellular Ca^{2+} in cylindrical coronary segments mounted either at L_0 or 1.2 L_0 axial length and subjected to 60 mm Hg. Data shown are means ± S.E.M of 10 coronary arteries from different animals.

$p < 0.05$, one-way ANOVA followed by Newman–Keuls' test). Active tension induced by Ca^{2+} at 60 mm Hg (28 ± 3 N m^{-1}, $n = 10$) was not different from those at 20 and 100 mm Hg.

Vascular sensitivity to extracellular Ca^{2+} was enhanced at 60 mm Hg when studied in cylindrical coronary segments mounted at 1.2 L_0 compared to L_0 (Fig. 6b). Thus, the pD_2 for Ca^{2+} in cylindrical segments studied at 1.2 L_0 and L_0 were 4.12 ± 0.04 ($n = 10$) and 3.95 ± 0.03 ($n = 10$), respectively ($p < 0.05$, unpaired *t*-test). Circumferential resting tension in segments mounted at L_0 (15 ± 1 N m^{-1}; $n = 10$) and 1.2 L_0 (14 ± 1 N m^{-1}; $n = 10$) were not different. Axial stretch of 20% did not influence the maximal contractile responses of coronary segments to Ca^{2+} (L_0: 29 ± 2 N m^{-1}; $n = 10$ vs. 1.2 L_0: 26 ± 2 N m^{-1}; $n = 10$).

Table 3
Inner radius and resting tension in cylindrical segments from porcine coronary arteries at different transmural pressures
Resting tension was calculated as $T = Pr_i$, where P is the transmural pressure and r_i under the assumptions related to Laplace's law for cylindrical segments. Results shown in the table correspond to Fig. 4a (I) and Fig. 6a (II). Data are expressed as means ± S.E.M. ($p < 0.05$, one-way ANOVA followed by Newman–Keuls' test).

	n	Inner radius (mm)	Resting wall tension (N m^{-1})
I (mm Hg)			
20	8	1.7 ± 0.1	5 ± 1
60	8	2.0 ± 0.1[a]	16 ± 1[a]
100	8	2.1 ± 0.1[a]	28 ± 1[ab]
II			
20	10	1.6 ± 0.1	4 ± 1
60	10	2.0 ± 0.1[a]	16 ± 1[a]
100	10	2.1 ± 0.1[a]	27 ± 2[ab]

[a]Compared to corresponding value obtained at 20 mm Hg.
[b]Compared to corresponding value obtained at 60 mm Hg.

4. Discussion

Our results suggest that preactivation, wall tension as well as geometry of the preparation influence the activity of voltage-gated Ca^{2+} channels on vascular smooth muscle cells, thereby modulating the sensitivity of porcine coronary arteries to both nifedipine and extracellular Ca^{2+}. Furthermore, axial wall tension present in cylindrical but not in ring preparations seems to be an important regulator of nifedipine-insensitive transmembrane Ca^{2+}-influx that may play a role for the tone and the vascular reactivity in large coronary arteries.

4.1. The influence of preactivation

The observed partial inhibition of prostaglandin $F_{2\alpha}$-induced contractions by high concentrations of L-type voltage-gated Ca^{2+} channel blocker indicates that the contractile action of prostaglandin $F_{2\alpha}$ in porcine coronary arteries involves mechanisms other than the activation of voltage-gated Ca^{2+} channels. Previous investigations have indicated that the action of prostaglandin $F_{2\alpha}$ is mediated predominantly by the release of Ca^{2+} from intracellular stores (Uski and Andersson, 1984) and Ca^{2+}-sensitization via protein kinase C (Kurata et al., 1993). In contrast, the effect of increasing the extracellular K^+ concentration is mediated by Ca^{2+}-influx via voltage-gated Ca^{2+} channels secondary to membrane depolarization (Somlyo and Somlyo, 1994). In addition to differences in transmembrane Ca^{2+}-influx via voltage-gated Ca^{2+} channels, the binding affinity for dihydropyridine-type Ca^{2+} antagonists has been

shown to be enhanced by membrane depolarization (Morel and Godfraind, 1987). The latter is more pronounced in prostaglandin $F_{2\alpha}$ compared to K^+-contracted arteries (Turner and Kozlowski, 1997). Therefore, the enhanced vascular sensitivity and the larger maximal inhibitory responses to nifedipine seen in K^+ compared to prostaglandin $F_{2\alpha}$-contracted preparations can be ascribed to the more pronounced membrane depolarization and concomitant activation of voltage-gated Ca^{2+} channels in K^+-activated preparations.

To avoid alteration of the release or action of endothelium-derived relaxing factors by K^+ depolarization (Kilpatrick and Cocks, 1994), the role of the endothelial cell layer in the determination of vascular sensitivity to nifedipine was investigated in prostaglandin $F_{2\alpha}$-contracted everted preparations. However, there are no L-type voltage-gated Ca^{2+} channels on vascular endothelial cells (Adams, 1994), and in the present study, we also found that the inhibitory effect of nifedipine was endothelium-independent.

Contractile responses induced by 25 mM K^+ showed a longer duration under isobaric compared to isometric conditions (Fig. 1). Membrane depolarization increases the affinity of voltage-gated Ca^{2+} channels to dihydropyridine-type Ca^{2+} antagonists in a time-dependent manner (Morel and Godfraind, 1987). Therefore, differences in the time-course of precontractile responses induced by partial depolarization could play a role for the differences in vascular sensitivity to nifedipine. However, if time effects had played a major role, vascular sensitivity to nifedipine should have been reduced under isometric compared to isobaric conditions, rather than enhanced as in the present study. In addition, the enhanced sensitivity of isometric preparations to nifedipine was also found in prostaglandin $F_{2\alpha}$-contracted segments, where there were no differences in the duration of precontractile responses. Thus, these observations suggest that differences in the time-course of K^+-induced precontractile responses in themselves cannot explain the differences in vascular sensitivity to nifedipine under isometric and isobaric conditions.

4.2. The influence of equilibrium wall tension

It has been suggested that wall tension influence the function of L-type voltage-gated Ca^{2+} channels in vascular smooth muscle (Schubert and Mulvany, 1999). In the present study, at 60 mm Hg, the vascular sensitivity to nifedipine as well as to Ca^{2+} was enhanced both in K^+ and prostaglandin $F_{2\alpha}$ when studied under isometric compared to isobaric conditions. Furthermore, an increase in resting wall tension increased the extracellular Ca^{2+}-dependent spontaneous resting tone as well as the vascular sensitivity to extracellular Ca^{2+}. These results suggest that differences in vascular sensitivity to nifedipine and extracellular Ca^{2+} under isobaric and isometric conditions can be explained by opposing changes of circumferential wall tension accompanying the vascular responses.

Mechanical stimuli were proposed to be involved in the regulation of the release of various endothelium-derived relaxant and contractile factors that may modulate vascular reactivity and sensitivity (Rubanyi et al., 1990). Our comparative investigations on everted coronary segments with and without endothelium indicated that differences in vascular sensitivity to cumulatively administered nifedipine under isometric and isobaric conditions were independent of the presence of endothelium. Thus, these results suggest that changes in circumferential wall tension affects the opening of L-type voltage-gated Ca^{2+} channels in the vascular smooth muscle cells.

4.3. The influence of vessel geometry

When the vascular sensitivity to nifedipine was compared in 25 mM K^+-contracted isometric preparations, wire-mounted rings were more sensitive to the inhibitory effect of the L-type voltage-gated Ca^{2+} channel blocker than cylindrical segments. In agreement with previous findings (Tankó et al., 1998a,b), the contractile response of coronary arteries to 25 mM K^+ was significantly larger in cylindrical segments compared to wire-mounted rings. Moreover, the vascular sensitivity to extracellular Ca^{2+} under the same experimental conditions was enhanced in cylindrical compared to ring preparations. Previous investigations have emphasized that the level of agonist-induced tone in the preparation is an important determinant of vascular sensitivity to a given relaxant agent (John et al., 1992). Accordingly, the decreased sensitivity to nifedipine in cylindrical compared to ring preparations could be ascribed to a larger transmembrane Ca^{2+}-influx in cylindrical segments.

Previous studies have indicated that geometry and thereby wall tension distribution is qualitatively different in wire-mounted rings and pressurized cylindrical segments (Dobrin, 1978; Cox, 1983; Lew and Angus, 1992; Falloon et al., 1995). While distending pressure acts in all directions, including axially, the axial length of a ring is independent of the distension applied by the uniaxially loading wires. Thus, these observations emphasize that wall tension in wire-mounted rings, in contrast to pressurized cylindrical segments, does not have an axial component.

The enhanced membrane excitability to depolarizing agents and the more prominent contractions to extracellular Ca^{2+} observed in cylindrical segments compared to wire-mounted rings could be due to differences in axial wall tension. An earlier study on small mesenteric arteries demonstrated that even when subjected to equivalent circumferential wall tensions, the resting membrane potential of vascular smooth muscle cells is significantly less negative in pressurized cylindrical segments compared to wire-mounted rings (Schubert et al., 1996). The authors sug-

gested that the differences in resting membrane potential could be due to a greater axial tension in the vessel wall of the pressurized cylindrical as compared to the ring preparations. This was also supported by the demonstration in the rat saphenous vein in situ that axial stretch of the vessel wall induces membrane depolarization resulting in an enhanced vascular sensitivity to contractile stimuli (Monos et al., 1993). Furthermore, the present study, in agreement with earlier findings by Garcia-Roldan et al. (1997) on rabbit epicardial coronary arteries, demonstrates that the extracellular Ca^{2+}-dependent spontaneous tone is modulated by the degree of axial extension in the vessel wall. Therefore, the involvement of an axial stretch-induced enhancement of membrane excitability and a consequent increase in transmembrane Ca^{2+} influxes may explain the enhanced reactivity to depolarizing agents in cylindrical segments compared to wire-mounted rings.

The vascular sensitivity was decreased to nifedipine but increased to extracellular Ca^{2+} in cylindrical segments as compared to wire-mounted rings. This discrepancy could be explained by the presence of transmembrane Ca^{2+}-influxes involving pathways other than the L-type voltage-gated Ca^{2+} channels. In support of this, the vascular sensitivity to Ca^{2+} studied in the presence of 125 mM K^+ increased with increasing resting circumferential or axial tension. In high K^+ solution, membrane potential of vascular smooth muscle cells is clamped and allow no modulation by stretch. Although a direct modulation of voltage-gated Ca^{2+} currents by stretch has been proposed in rat cerebral arteries (McCarron et al., 1997), this mechanism has not been confirmed in porcine coronary arteries (Davis et al., 1992a). However, Davis et al. (1992a) have described the existence of Ca^{2+} currents resistant to blockers of voltage-gated Ca^{2+} channels (Davis et al., 1992a,b). Moreover, in a previous report from our laboratory (Frøbert et al., 1996), we found that a 20% increase in axial length decreased the sensitivity of the porcine coronary artery to nifedipine. This finding, together with results of the present study suggest that 20% axial stretch enhances transmembrane Ca^{2+}-influx through nifedipine-insensitive channels. Therefore, the decreased sensitivity of cylindrical segments to nifedipine as compared to wire-mounted rings could also involve an increased activation of nifedipine-insensitive Ca^{2+} currents.

Pressure/stretch-induced membrane depolarization and consequent opening of voltage-gated Ca^{2+} channels are important determinants of the myogenic activity (Schubert and Mulvany, 1999). While pressure-induced membrane depolarization has been described in cylindrical preparations from various vascular beds, this phenomenon could not be demonstrated when rings were stretched over wires (Harder et al., 1983; Dunn et al., 1994). The extracellular Ca^{2+} and resting tension-dependent spontaneous tone of porcine coronary arteries seen in cylindrical segments could not be revealed when the vessels were studied as wire-mounted rings. Several explanations such as the lack of axial extension and radial compression as well as damage occurring to smooth muscle when stretched over wires has been proposed (Harder, 1984; Osol, 1995). Nifedipine-insensitive stretch-activated cation channels are important initiators of pressure/stretch-induced membrane-depolarization (Davis et al., 1992a; Setoguchi et al., 1997; Takenaka et al., 1998). Therefore, the lacking resting tone in wire-mounted rings could be explained by a reduced activation of nifedipine-insensitive stretch-activated cation channels in these preparations.

To provide direct experimental evidence for the apparent involvement of nifedipine-insensitive stretch-activated cation channels, a specific blocker of these channels is needed (Caldwell et al., 1998). Gadolinium has been shown to block these channels in the concentration range of 10–20 μM (Yang and Sachs, 1989). However, single cell studies on porcine coronary arteries have shown that gadolinium completely blocks voltage-gated Ca^{2+} channels already in the nanomolar range (Song et al., 1992) and therefore cannot be applied as a specific blocker of stretch-activated channels in porcine coronary arteries.

4.4. Conclusions

The results of the present study suggest that the vascular sensitivity of porcine coronary arteries to nifedipine and extracellular Ca^{2+} in vitro is influenced by preactivation, wall tension as well as the geometry of the preparation. Furthermore, axial wall tension present in cylindrical but not in ring preparations seems to be an important regulator of nifedipine-insensitive transmembrane Ca^{2+}-influx. The Ca^{2+}-influx via the aforementioned channels appears to be a prerequisite for the tone and a determinant of vascular reactivity in large coronary arteries.

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