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A new experimental approach in endothelium-dependent pharmacological investigations on isolated porcine coronary arteries mounted for impedance planimetry

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1 The aim of this study was to investigate whether the balloon-based impedance planimetry technique could be a useful tool in endothelium-dependent investigations.

2 Porcine large coronary arteries contracted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 10 μM) did not relax to bradykinin (0.1 nM – 0.1 μM), but did relax to sodium nitroprusside (SNP, 10 μM). However, after eversion of the segments, bradykinin induced relaxations with pD_2 values and maximal responses of 8.78 ± 0.09 and $75 \pm 2\%$ ($n = 6$), respectively.

3 Incubation with captopril (1 μM) did not reveal a relaxation to bradykinin in the normal vessel configuration and had no influence on the concentration-relaxation relationship in everted segments.

4 Lowering the luminal pressure in contracted segments from 131 ± 5 mmHg (isometric, $n = 5$) to 60 mmHg (isobaric, $n = 5$) did not facilitate the action of bradykinin.

5 Eversion of segments did not influence the concentration-response relationship for K^+ (4.7 – 125 mM), $PGF_{2\alpha}$ (0.3 – 30 μM), and SNP (30 nM – 30 μM), although the time-courses of responses were faster when the agents were added from the intimal compared to the adventitial side of the preparation.

6 In the same everted segment contracted with $PGF_{2\alpha}$, the concentration-response relationship for bradykinin was not different under isometric and isobaric conditions.

7 These results indicate that, (1) reduced endothelium-dependent relaxations to adventitially administered substances can be ascribed to a diffusion barrier in the vessel wall, while enzymatic degradation, luminal pressure and precontractile responses seem not to play a role, (2) impedance planimetry applied to everted cylindrical segments could be a useful experimental approach in pharmacological studies of endothelium-dependent responses under isobaric and isometric conditions.

Keywords: Bradykinin; diffusion barrier; endothelium; eversion; impedance planimetry; isobaric and isometric conditions; large coronary artery; luminal pressure; pig; time-course

Abbreviations: A23187, 6S-[6α(2S*,3S*),8β(R*),9β,11α]-5-(methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-oxo-2-(1H-pyrrol-2-yl)ethyl]-1,7-dioxaspirol[5,5]-undec-2-yl]methyl]-4-benzoxazolecarboxylic acid; EDTA, ethylenediaminetetraacetic acid; K^+, potassium; NO, nitric oxide; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; SNP, sodium nitroprusside

Introduction

The pharmacodynamic properties of both the vascular smooth muscle and the endothelium depend on the mechanical conditions to which the vessel is subjected (Osol, 1995; Rubanyi *et al*., 1990). Cumulative evidence from small artery studies suggest that the use of cylindrical segments offer advantages over wire-mounted rings in *in vitro* investigations (Dunn *et al*., 1994; Buus *et al*., 1994; Falloon *et al*., 1995). Cylindrical arterial segments maintain normal geometry (Falloon *et al*., 1995) and myogenic activity (Dunn *et al*., 1994) resulting in a significantly higher reactivity and sensitivity to vasoactive stimuli compared with that of wire-mounted rings. Pressurizing techniques, such as the pressure-myograph for small arteries (Halpern *et al*., 1984), are therefore, used more and more extensively in studies on the pharmacological and physiological properties of isolated arteries. Despite these considerable advantages of using cylindrical segments, studies on medium-sized arteries are still mainly carried out on wire-mounted rings.

The impedance planimetry technique is a multi-functional tool based on a hydraulically distendable balloon by which controlled and axisymmetric deformations can be carried out in tubular organs, while obtaining simultaneous measurements of luminal cross-sectional area and pressure (Gregersen *et al*., 1988). The technique, therefore, has already been used extensively in studies on the passive mechanical properties of the gastrointestinal (e.g. Rao *et al*., 1995) and the urinary tracts (Lose & Colstrup, 1990) as well as of arteries (Storkholm *et al*., 1997). Frøbert *et al*. (1996) proposed that the impedance planimetry technique could be a useful supplement to the well-established wire (isometric) and conventional pressure (isobaric) techniques for the study of medium-sized arteries. A systematic evaluation of an improved, multi-mode (isobaric and isometric) version of the impedance planimetry probe has recently been demonstrated in basic endothelium-independent pharmacodynamic investigations on porcine epicardial coronary arteries (Tankó *et al*., 1998). Whether the technique could be a useful tool in endothelium-dependent investigations as well has remained unclarified so far. Eversion of vascular segments have earlier been applied in the investigation of a functional adventitial barrier (Wang *et al*., 1998). In addition, this experimental model has been proposed for functional investigations on vascular smooth muscle in porcine coronary arteries (Makujina *et al*., 1995). However, it has not been

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investigated whether the everted preparation could be a useful approach for the study of endothelium-dependent responses in medium-sized arteries.

Morphological investigations have shown earlier that the endothelial cell layer is intact in arterial segments that have undergone careful probe insertion (Frøbert *et al*., 1996). In contrast, our preliminary investigations showed that bradykinin administered from the adventitial side could not evoke considerable relaxant response in porcine coronary arteries when studied with the impedance planimetry technique.

Therefore, the purpose of the present study was to clarify whether the lack of relaxation to adventitially administered bradykinin could be ascribed to one of the following possibilities: (1) irreversible functional damage of the endothelium caused by the probe, (2) degradation of bradykinin by the angiotensin-converting enzyme, (3) endothelial dysfunction induced by higher luminal pressure in isometrically contracted vessels or (4) the presence of a diffusion barrier in the vessel wall. To further validate this experimental model, the influence of eversion on vascular smooth muscle function was evaluated by comparing the concentration-response relationship for various contractile and relaxant agents in the two configurations of the same coronary artery.

Methods

Porcine epicardial coronary arteries

Hearts from Danish Landrace-Yorkshire pigs weighing 80–90 kg were obtained at a local abattoir and transported to the laboratory in 5°C physiological saline solution (PSS, composition described below) previously aerated with 5% CO_2 in 95% O_2. Experiments started 60–90 min after sacrifice of the pigs. The proximal 3 cm long segment of the left anterior descending coronary artery with an inner diameter of 3–3.5 mm was dissected and trimmed of the adherent fat and connective tissue while submerged in oxygenated ice-cold PSS. The arterial segments were then mounted on the cannulas of a specially designed vessel holder, fastened with 4–0 ligatures, and placed in a 50 ml organ bath filled with PSS. The organ bath was continuously bubbled with 5% CO_2 in 95% O_2 at 37°C to give a pH of 7.4. Before the probes were gently and concentrically inserted into the vessel lumen, the segments were allowed to equilibrate 60 min in the organ bath.

The impedance planimetry probe and experimental setup

The impedance planimetry probe used in the present study has been described previously in detail (Tankó *et al*., 1998). In brief, the probe consists of a specially designed four-electrode impedance measuring system inside a 25 mm long, thin-walled (50 μm), non-conducting polyurethane balloon (diameter 6 mm) mounted on a 70 mm long 3 Fr probe. After inserting the probe in the vessel lumen, the cylindrical balloon was distended with electrically conducting saline solution (0.09% NaCl). Previous investigations showed that a basal distending pressure of 60 mmHg ensures optimal vascular smooth muscle function in these preparations both under isometric and isobaric conditions (Tankó *et al*., 1998). Therefore, all investigations in the present study were carried out at basal conditions corresponding to this luminal pressure.

The combined isobarbic and isometric applicability of the impedance planimetry technique lies in its ability to measure luminal cross-sectional area and pressure simultaneously. If the vessel is subjected to a constant luminal pressure and vascular responses are recorded 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 of the infusion channel) and responses are recorded as changes in luminal pressure, the system mode is isometric.

The balloon cross-sectional area was estimated by measuring the electrical impedance of the saline solution inside the balloon using two electrodes for excitation and two electrodes for detection as described previously (Harris *et al*., 1971). Briefly, when a current (I) is induced in a conductor by the excitation electrodes, the potential difference (V) between the detection electrodes is $V = I\,d\,\rho^{-1}A^{-1}$, where d is the distance between the detection electrodes, and ρ is the conductivity of the conductor, and A is the cross-sectional area of the cylindrical balloon. In the impedance measuring system, I, d and ρ are constants, and consequently V is inversely proportional to A. Direct proportionality was obtained by means of the data acquisition software (Supermingo®, GateHouse, Aalborg, Denmark). Calibration of the cross-sectional area measuring system was performed at 37°C in a solid polyphenolenoxide block with holes of known cross-sectional area ranging from 7.06 mm^2 to 28.26 mm^2.

Pressure inside the balloon and thereby in the vessel lumen was estimated by a low compliance external pressure transducer (Uniflow™, Baxter, Irvine, CA, U.S.A.) coupled to the infusion channel of the probe. Pressure transducer calibration was performed by applying pressures of 0 and 100 mmHg by a level container. During the experiments, the transducer was set at fluid level of the organ bath.

On-line recordings of experimental investigations were analysed quantitatively (as a function of time) by the MotAn® data analysis software (GateHouse, Aalborg, Denmark).

Experimental protocols

To investigate whether the lack of relaxant response to bradykinin can be ascribed to an irreversible functional damage of the endothelium caused by the probe insertion, the effect of cumulatively administered (0.1–100 nM) bradykinin was studied first in the normal (intimal surface inside the lumen) then in the everted (intimal surface outside the lumen) configuration of the same coronary artery. After obtaining a reproducible contractile response to 125 mM K^+ under isometric conditions, the vessel was activated with 10 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). When the contraction reached steady-state, bradykinin was added cumulatively into the organ bath. Having finished the concentration-response experiment in the normal configuration, the vessel was returned to the initial relaxed condition by repeated washing with PSS, the balloon was then completely deflated, and the probe was gently pulled out of the vessel lumen. The vessel was demounted and carefully everted by gently rolling out the distal part of the segment through the proximal lumen while keeping it relaxed in warm oxygenated PSS (within 5 min). Subsequently, the vessel was remounted, set at the same distending pressure of 60 mmHg and allowed to equilibrate for 60 min. Thereafter, according to the above protocol, a new concentration-response curve for bradykinin was obtained. Finally, we evaluated the effect of endothelium removal on vascular responses to cumulatively added bradykinin. Endothelium was removed by gently and systematically rubbing the intimal surface of the everted cylindrical segment using moistened cotton wool fixed on a thin wooden stick. The nitric oxide (NO)-donor sodium nitroprusside (SNP, 10 μM) was used to evaluate the relaxant ability of vascular smooth muscle.

To investigate whether a rapid degradation of the substance by the angiotensin-converting enzyme could be involved in the mechanisms leading to the lack of relaxation to adventitially administered bradykinin, the segments were contracted with 10 μM $PGF_{2\alpha}$ and a first concentration-response curve for bradykinin was obtained. After a 60 min preincubation with an inhibitor of the enzyme, captopril (1 μM) (Thind, 1990), a second concentration-response curve for bradykinin was

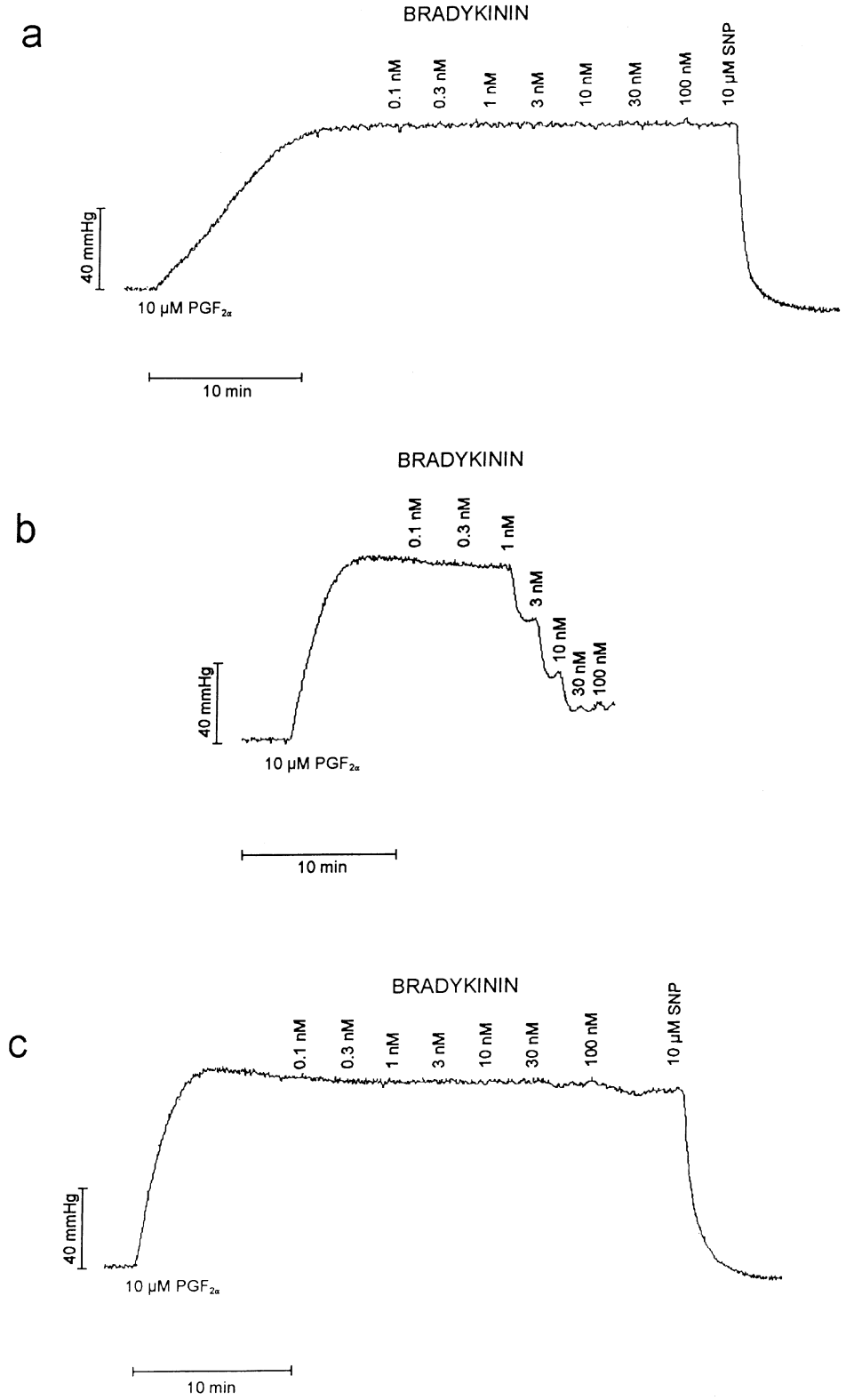

Figure 1 Original trace recordings showing the effect of cumulatively (0.1 – 100 nM) administered bradykinin in the normal vessel configuration (a), in the everted vessel configuration (b) and in the everted vessel configuration after mechanical removal of the endothelium (c) in the same coronary artery segment contracted with 10 μM prostaglandin $F_{2\alpha}$ under isometric conditions at 60 mmHg. The nitric oxide (NO)-donor, sodium nitroprusside (SNP, 10 μM), was used as a control for the relaxant ability of vascular smooth muscle.

constructed. During the first 30 min the balloon was deflated thereby allowing access for captopril from both the adventitial and the luminal sides. With the same purpose, the concentration-response relationship for bradykinin was studied in everted coronary artery segments contracted with 10 μM $PGF_{2\alpha}$ without and after a 60 min preincubation with 1 μM captopril. Parallel time control curves for bradykinin were also obtained by constructing consecutive concentration-response curves in everted preparations.

To investigate whether the explanation could be a reversible dysfunction of the endothelial cells in response to the higher luminal pressure in isometrically contracted arteries, the effect of cumulatively added bradykinin was evaluated under isobaric conditions with reference to the isometric conditions in 10 μM $PGF_{2\alpha}$-contracted coronary segments. After constructing the concentration-response curve under isometric conditions, the vessel was relaxed and allowed to equilibrate at 60 mmHg for 60 min. Thereafter, the preparation was contracted under isobaric conditions with 10 μM $PGF_{2\alpha}$ and bradykinin was added cumulatively into the organ bath. The ability of vascular smooth muscle to relax in response to a NO donor was verified by the addition of 10 μM SNP. After eversion of the segment, the presence of the endothelium was examined by adding 100 nM bradykinin into the organ bath.

To investigate whether the lack of relaxation in the coronary segments to adventitially administered bradykinin is a specific phenomenon to this substance, the effect of substance P (0.3 μM) and the receptor-independent calcium ionophore A23187 (1 μM) in the normal and everted configurations of the same coronary artery was also compared.

The influence of eversion on the vascular sensitivity and maximal response of vascular smooth muscle to various contractile and relaxant stimuli was evaluated by comparing the concentration-response relationship for potassium (K^+, 4.7–125 mM), $PGF_{2\alpha}$ (0.3–30 μM), and SNP (30 nM–30 μM) in the normal and everted configurations of the same coronary artery. The relaxant effect of SNP was investigated on 10 μM $PGF_{2\alpha}$-induced contractions. A 60 min period was allowed for the everted preparation before inducing contraction with 10 μM $PGF_{2\alpha}$. Reproducibility of concentration-response curves to K^+, $PGF_{2\alpha}$, and SNP were also evaluated in separate experiments applying the same wash-out period of 60 min.

In a separate series, the time course of contractile response to 125 mM K^+ and 10 μM $PGF_{2\alpha}$ as well as of relaxant responses to 10 μM SNP administered from the adventitial and the intimal sides of the same coronary segment were also compared. Starting point for time recording was the moment of adding the agent into the organ bath. Dynamics of responses were expressed as the time required to reach half maximal response ($t_{1/2}$).

Finally, we investigated whether the mode of recording vascular responses (isometric as compared to isobaric) influences the vascular sensitivity and maximal response of the coronary artery to cumulatively added bradykinin. Concentration-response curves to bradykinin obtained at the same basal pressure of 60 mmHg under isobaric and isometric conditions were compared in the same everted segments contracted with 10 μM $PGF_{2\alpha}$. A 60 min equilibration period was allowed in between the consecutive stimulations. The order of isometric or isobaric test modes was randomly alternated in the preparations.

Solutions and drugs

The composition of the PSS was (in mM): NaCl 100, $NaHCO_3$ 15, KCl 4.7, $MgCl_2$ 10, $NaHPO_4$ 1.2, $2H_2O$ 1.5, $CaCl_2$, glucose 11.1 and ethylenediaminetetraacetic acid (EDTA) 0.027. 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 bradykinin HCl and substance P (Sigma, St. Louis, MO, U.S.A.), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Dinoprost, Upjohn, Puurs, Belgium), sodium nitroprusside dihydrate (SNP, Merck, Darmstadt, Germany), and 6S-[6α(2S*,3S*),8β(R*),9β,11α]-5-(Methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-oxo-2-(1H-pyrrol-2-yl)ethyl]-1,7-dioxaspirol[5,5]-undec-2-yl]methyl]-4-benzoxazolecarboxylic acid (A23187, Calbiochem-Behring Corporation, La Jolla, CA, U.S.A.). Bradykinin, substance P and SNP were dissolved in twice distilled water, A23187 in dimethyl-sulphoxide, and $PGF_{2\alpha}$ in 96% alcohol. The final vol% of 96% alcohol and dimethyl-sulphoxide were under 0.01% in the organ bath and had no direct effect on active vessel tone.

Analysis and statistics

The mechanical responses of the vessels under isometric conditions were measured as changes in luminal pressure and expressed as active wall tension ΔT, which is the increase in pressure, ΔP, multiplied by the inner radius of the segment, r_i (Laplace relation for cylindrical segments). The mechanical responses under isobaric conditions were measured as changes in cross-sectional area and expressed as active wall tension, ΔT, which is the constant pressure P multiplied by the decrease in inner radius, r_i. Using a computer programme (GraphPad, Institute for Scientific Information, CA, U.S.A.), 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.

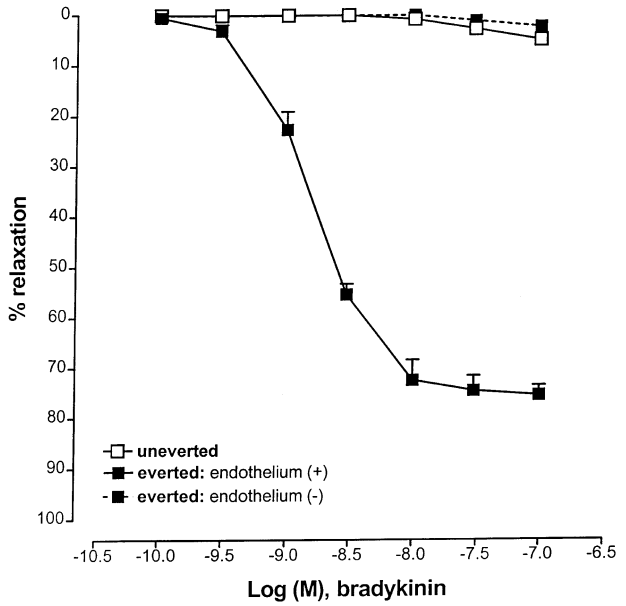

Figure 2 (a) Effect of cumulatively administered bradykinin (0.1–100 nM) in the normal and everted (+ and − endothelium) configurations of the same coronary artery segment contracted with 10 μM prostaglandin $F_{2\alpha}$. Values shown are means ± s.e.mean of six arteries from different animals.

The results are expressed as means ± s.e.mean, and the response curves presented on a semi-logarithmic scale. Differences between means were analysed using Student's two-tailed paired *t*-test. Level of significance was $P<0.05$.

Results

Responses to bradykinin

Under isometric conditions, 10 μM $PGF_{2\alpha}$ increased tension with 22 ± 2 Nm^{-1} ($n=6$) which was $65\pm6\%$ ($n=6$) of the corresponding maximal response to 125 mM K^+. These contractile responses were stable for 45–60 min.

Administered cumulatively from the adventitial side of the coronary artery, bradykinin (0.1 nM–0.1 μM) induced no relaxation (Figures 1a and 2). In two experiments, small and transient contractions were observed to higher concentrations (30–100 nM) of bradykinin. In the rest of the experiments ($n=4$), bradykinin administered from the adventitial side induced small relaxations, which were less than 10% of the contractile responses induced by 10 μM $PGF_{2\alpha}$. These preparations relaxed $103\pm3\%$ ($n=6$) to the NO donor, SNP (10 μM) (Figure 1a).

Eversion of the intimal surface of porcine coronary artery segment did not significantly change the contractile responses of vascular smooth muscle (Figure 1a–c). Thus, increases in tension induced by 10 μM $PGF_{2\alpha}$ in the normal and everted configurations of the coronary segments were 22 ± 2 Nm^{-1} and 23 ± 1 Nm^{-1}, respectively ($n=6$). In contrast to arterial segments mounted in the normal configuration, everted preparations showed concentration-dependent relaxations to cumulatively administered bradykinin (Figures 1b and 2) with a pD_2 value and maximal relaxation of 8.78 ± 0.09 ($n=6$) and $75\pm2\%$ ($n=6$), respectively. In these everted preparations, mechanical endothelial cell removal abolished the relaxations induced by bradykinin, but did not affect relaxations to 10 μM SNP (Figures 1c and 2). The maximal response to 10 μM SNP was $102\pm2\%$ ($n=6$).

Inhibition of the angiotensin-converting enzyme

Inhibition of the angiotensin-converting enzyme with 1 μM captopril did not facilitate the relaxant action of bradykinin in coronary arteries mounted in the normal configuration (Figure 3). Thus, the maximal responses to cumulatively (0.1–100 nM) administered bradykinin were $4\pm1\%$ and $3\pm1\%$, in the absence and presence of 1 μM captopril, respectively ($n=6$).

In everted coronary artery segments contracted with 10 μM $PGF_{2\alpha}$, the concentration-response relationship for bradykinin without and after incubation with 1 μM captopril (60 min) showed no significant differences (Figure 3); the pD_2 values were 8.81 ± 0.09 and 8.72 ± 0.08 and the maximal responses $96\pm7\%$ and $93\pm6\%$, respectively ($n=6$). The increases in tension to 10 μM $PGF_{2\alpha}$ were 19 ± 3 Nm^{-1} (first contraction, $n=6$) and 19 ± 2 Nm^{-1} (second contraction, $n=6$).

In parallel time control experiments, concentration-response curves to bradykinin were found reproducible in everted coronary segments with pD_2 values and maximum responses of 9.03 ± 0.12 and $93\pm8\%$, respectively, in a first control curve ($control_1$, $n=6$) and 8.94 ± 0.08 and $95\pm6\%$, respectively, in a second concentration-response curve ($control_2$, $n=6$). Agonist-induced active tensions to 10 μM $PGF_{2\alpha}$ were 19 ± 2 Nm^{-1} ($control_1$, $n=6$) and 20 ± 2 Nm^{-1} ($control_2$, $n=6$).

Effect of luminal pressure on bradykinin relaxation

Under isometric and isobaric conditions, the luminal pressure in the 10 μM $PGF_{2\alpha}$-contracted coronary segments was 131 ± 5 mmHg and 60 ± 0 mmHg ($n=5$, $P<0.001$, paired *t*-test), respectively. Changes in tension induced by 10 μM $PGF_{2\alpha}$ under isometric and isobaric conditions were 20 ± 3 Nm^{-1} and -11 ± 2 Nm^{-1} ($P<0.001$, $n=5$, paired *t*-test), respectively. Adventitially administered bradykinin did not induce significant relaxations either under isometric ($3\pm1\%$, $n=5$) or under isobaric conditions (0%, $n=5$). SNP (10 μM) effectively relaxed the segments under both experimental conditions with maximal responses of $99\pm3\%$ (isometric, $n=5$) and $89\pm4\%$ (isobaric, $n=5$). After everting the intimal surface of these coronary segments, the presence of endothelium was indicated by a maximal relaxant response of $84\pm6\%$ ($n=5$) to 100 nM bradykinin.

Responses to substance P and A23187

Increases in tension induced by 10 μM $PGF_{2\alpha}$ in the normal and everted configurations were 21 ± 3 Nm^{-1} and 22 ± 2 Nm^{-1}, respectively ($n=6$). Under isometric conditions, 0.3 μM substance P caused only small relaxations of $6\pm1\%$ ($n=6$) when administered from the adventitial side. In contrast, it caused relaxations of $84\pm6\%$ ($P<0.001$, $n=6$, paired *t*-test) when applied in the everted configuration of the preparation.

Increases in tension induced by 10 μM $PGF_{2\alpha}$ in the normal and everted configurations were 19 ± 3 Nm^{-1} and 20 ± 2 Nm^{-1}, respectively ($n=6$). The calcium ionophore A23187 induces receptor-independent endothelium-dependent relaxations in porcine coronary arteries. Similarly to substance P, A23187 also had modest relaxant effect in the coronary artery segments mounted in the normal configuration, while causing relaxation in the everted configuration. Thus, 1 μM

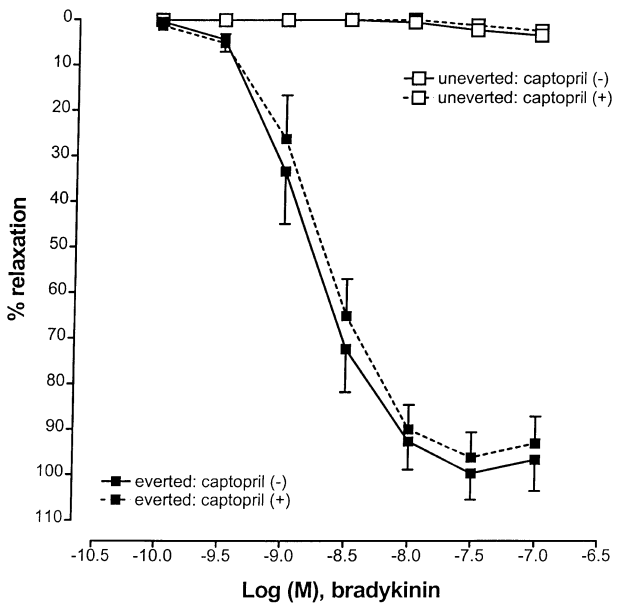

Figure 3 Effect of cumulative (0.1–100 nM) administered bradykinin in the absence and presence of 1 μM captopril in the same coronary artery segment mounted in the normal configuration and contracted with 10 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Figure also shows the concentration-response relationship for bradykinin in the absence and presence of 1 μM captopril in the same everted coronary artery segment contracted with 10 μM $PGF_{2\alpha}$. Values shown are means ± s.e.mean of six arteries from different animals.

A23187 relaxed the $PGF_{2\alpha}$-contracted segments in the normal and everted configurations with maximal responses of $4\pm1\%$ and $79\pm8\%$, respectively ($P<0.001$, $n=6$, paired t-test).

Comparison of the contractile and relaxant properties in normal and everted coronary arteries

Eversion of coronary arterial segments did not change the concentration-response relationship for K^+ (4.7–125 mM), $PGF_{2\alpha}$ (0.3–30 μM) and SNP (30 nM–30 μM). The pD_2 values and maximal responses for these substances in the normal and everted configurations of the same coronary segment are shown in Table 1. Concentration-response curves to K^+, $PGF_{2\alpha}$, and SNP were all reproducible after a 60 min wash-out period. The pD_2 values and maximal responses are indicated in Table 1.

The time-course of maximal contractile responses to 125 mM K^+ and 10 μM $PGF_{2\alpha}$ as well as of relaxant responses to 10 μM SNP showed significant differences when induced from the intimal compared to the adventitial side of the segment (Table 2). Onset of the response was more rapid and the time required to reach half maximal response was significantly shorter when these substances were administered from the intimal compared to the adventitial side of the preparation.

Responses to bradykinin under isobaric and isometric conditions

In the same everted coronary segments, changes in tension induced by 10 μM $PGF_{2\alpha}$ were 21 ± 3 Nm^{-1} and -10 ± 2 Nm^{-1} under isometric and isobaric conditions, respectively ($P<0.001$, $n=7$, paired t-test). Cumulatively administered bradykinin induced relaxant response both under isobaric and isometric conditions (Figure 4a,b) with no significant differences in the vascular sensitivity or maximal response to the substance (Figure 4c). Thus, pD_2 values for bradykinin under isobaric and isometric conditions were 8.51 ± 0.07 and 8.66 ± 0.06 ($n=7$, $P=0.11$, paired t-test), respectively, while maximal responses were $86\pm5\%$ and $92\pm3\%$ ($n=7$), respectively.

Table 1 The effect of repetition and eversion on the concentration-response relationship for potassium (K^+), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and sodium nitroprusside (SNP)

		n	pD_2	*Maximal response* (Nm^{-1})
K^+	$control_1$	6	1.66 ± 0.02	31 ± 2
	$control_2$	6	1.63 ± 0.03	30 ± 2
	normal	6	1.64 ± 0.02	32 ± 2
	everted	6	1.69 ± 0.03	31 ± 2
$PGF_{2\alpha}$	$control_1$	3	5.03 ± 0.04	21 ± 2
	$control_2$	3	5.09 ± 0.05	22 ± 3
	normal	6	5.10 ± 0.03	23 ± 3
	everted	6	5.19 ± 0.05	23 ± 4
SNP	$control_1$	6	6.11 ± 0.13	$103\pm3\%$
	$control_2$	6	6.07 ± 0.16	$107\pm3\%$
	normal	6	6.00 ± 0.10	$106\pm2\%$
	everted	6	6.02 ± 0.15	$110\pm4\%$

Data are expressed as means±s.e.mean. The experiments were performed in the same porcine coronary arteries under isometric conditions at a basal pressure of 60 mmHg. In the SNP experiments, increases in tension induced by 10 μM $PGF_{2\alpha}$ were 21 ± 3 Nm^{-1} in a first concentration-response curve ($control_1$), 22 ± 3 Nm^{-1} in a second concentration-response curve ($control_2$), 18 ± 3 Nm^{-1} in the normal configuration (normal) and 20 ± 4 Nm^{-1} in the everted configuration (everted). $pD_2=-\log(EC_{50})$, where EC_{50} is the concentration giving half-maximal response.

Discussion

The main findings of the present study are as follows: First, adventitially administered bradykinin cannot evoke relaxant responses *via* the endothelium in isolated porcine large coronary artery segments when studied by impedance planimetry, apparently, due to the presence of an effective diffusion barrier in the vessel wall limiting the access to the

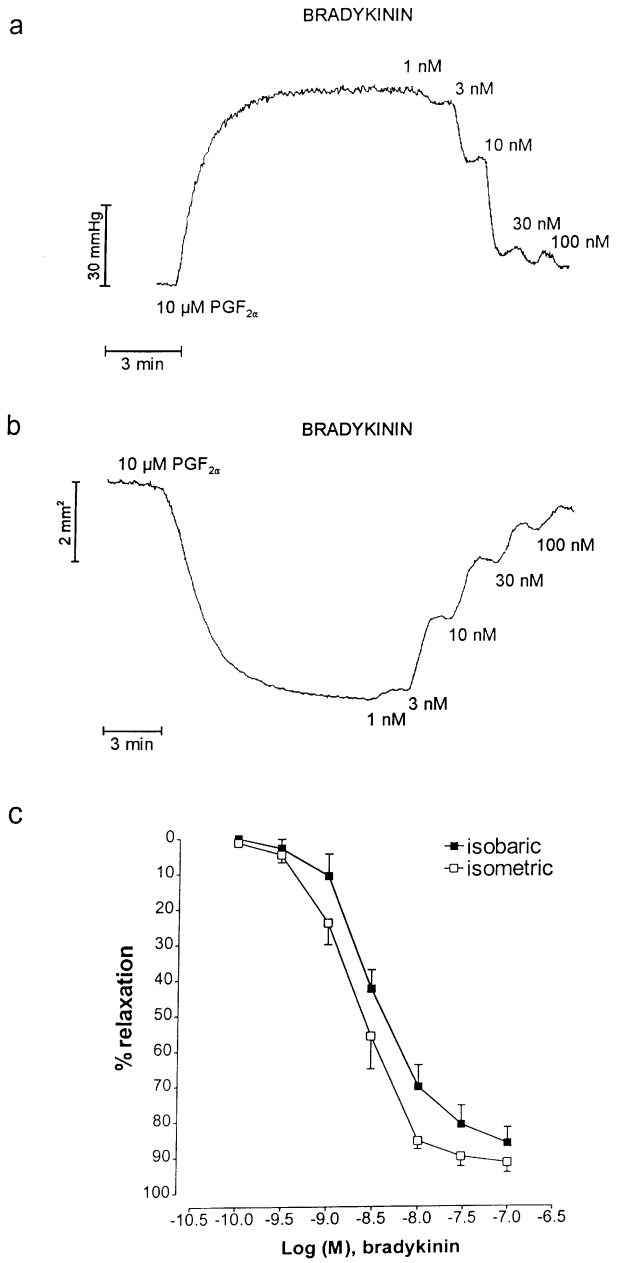

Figure 4 Original trace recordings showing the effect of cumulatively added bradykinin in the same everted coronary artery segment contracted with 10 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) under isometric (a) and isobaric (b) conditions. (c) Comparison of the concentration-response relationship for bradykinin in the same everted coronary artery segment contracted with 10 μM $PGF_{2\alpha}$ under isometric and isobaric conditions. Values shown are means±s.e.mean of seven arteries from different animals.

Table 2 Time-course of responses to 125 mM potassium (K^+), 10 mM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 10 mM sodium nitroprusside (SNP)

		n	$t_{1/2}$ (s)	*Maximal response* (Nm^{-1})
K^+	normal	6	53±3	34±2
	everted	6	27±3*	30±2
$PGF_{2\alpha}$	normal	6	346±32	22±3
	everted	6	164±24*	22±4
SNP	normal	6	70±5	103±2%
	everted	6	32±4*	106±3%

Data are expressed as means±s.e.mean. $t_{1/2}$ indicates the time required to reach half-maximal response. The experiments were performed in the normal and everted configurations of the same coronary artery segment under isometric conditions as a basal pressure of 60 mmHg. In the SNP experiments, active tension induced by 10 μM $PGF_{2\alpha}$ was 23±2 Nm^{-1} both in the normal and the everted configurations of the coronary segments. *Significant difference ($P<0.001$, paired *t*-test) compared to the corresponding 'normal' value.

intact endothelial cell layer. Enzymatic degradation, luminal pressure and contractile responses seem not to play a major role in the lack of effect of bradykinin. Second, the impedance planimetry technique in combination with everted cylindrical segments from medium-sized arteries is a useful experimental approach to study endothelium-dependent responses under both isobaric and isometric conditions in medium-sized arteries.

Morphological studies have indicated an intact endothelial cell layer in porcine large coronary arteries that have undergone careful probe insertion (Frøbert *et al*., 1996). In addition, proper match of vessel and probe size (approximately 3 : 1) is required. Therefore, the present observation of no responses in the normal, in contrast to the effective relaxation seen in the everted configuration, appears to be due to factors other than the irreversible functional damage of the endothelial cell layer.

Based on the above findings, it was assumed that the access of bradykinin is impaired to its endothelial receptors. In this regard, a major limitation can be a rapid enzymatic degradation within the vessel wall during the transmural diffusion of the substance. The angiotensin-converting enzyme, known also to be responsible for the enzymatic degradation of bradykinin (Hornig & Drexler, 1997), was shown to be extensively distributed in all layers of the vessel wall of large and medium-sized arteries, including coronary arteries. Enzymatic activity was found to be the highest in the endothelium, but it was considerable in the media and the adventitia as well (Okunishi *et al*., 1987; Sim 1990; Arnal *et al*., 1994; Diet *et al*., 1996). However, Graf *et al*. (1991) measured the rate of bradykinin degradation in isolated large coronary arteries equilibrated in organ baths. They found that the half-life of the peptide was 27 min, much longer than it takes to induce vasodilation (2–3 min). The present findings that the inhibitor of the angiotensin-converting enzyme, captopril (Thind, 1990), does not change the relaxant action of bradykinin support that a rapid degradation of the peptide does not play a major role in functional *in vitro* studies. Furthermore, a rapid degradation cannot explain the lack of relaxation to bradykinin when it is administered from the adventitial side of the preparation.

Another explanation could be the dysfunction of smooth muscle to relax to endothelium-derived relaxant factors. However, this does not seem to be the case, as the NO donor SNP effectively relaxed the coronary artery segments, at the same time suggesting that the lack of relaxant response to bradykinin is due to the lack of release of relaxant factors from the endothelium.

The lack of release of endothelium-derived relaxant factors could be due to a reversible endothelial cell dysfunction induced by the higher luminal pressure acting on the intimal surface of the isometrically contracted vessels. The probability of the contribution of this factor is supported by both *in vitro* (Rubanyi *et al*., 1991) and *in vivo* (Vanhoutte, 1996) observations showing that luminal pressure may reduce the production and/or action of endothelium-derived NO and endothelium-derived hyperpolarizing factor. Such endothelial dysfunction may occur in the course of the hypertensive process as well (Vanhoutte, 1996). To evaluate this aspect, the effect of cumulatively added bradykinin was studied also under isobaric conditions where luminal pressure remains constant during the course of the contraction. However, our results indicated that carrying out the evaluation under these conditions did not facilitate the effect of bradykinin to evoke relaxation, suggesting that pressure appears not to play a major role. In support of this statement, cannulated small arteries pressurized to 60 mmHg were shown to allow for the study of endothelium-dependent responses under isobaric conditions (Boyle & Maher, 1995). Thus, providing that endothelium-dependent substances reach their receptors, bradykinin should have induced relaxation in our experiments as well. In this context, it is interesting to point out that in everted arterial segments, similarly as in wire-mounted rings, the endothelium experience no considerable radial pressure. Therefore, the mechanical state of the endothelium is only a function of the circumferential and axial strains to which the vessel wall is subjected.

The lack of relaxation to adventitially administered bradykinin could not be explained by enzymatic degradation of the substance. Cohen *et al*. (1984) suggested that the thick (approximately 400 μm) wall of these large epicardial coronary arteries, consisting of a dense collagenous adventitia, multilayered smooth muscle, and compact external and internal elastic laminae (Rhodin, 1980), may act as an effective diffusion barrier in the vessel wall for endothelium-dependent substances administered from the adventitial side. This concept was supported by their observations that in canine large coronary artery segments with intact endothelium, intraluminal, but not extraluminal, acetylcholine, adenosine diphosphate, or thrombin caused relaxation (Cohen *et al*., 1984). Angus *et al*. (1983) reported that adventitially administered acetylcholine induced small relaxant responses in canine femoral arteries *in situ*, but the agent was 50–100 times less potent by this route compared with intra arterial-infusion. The lack of response to adventitially administered substance P and the calcium-ionophore A23187 in the present study also emphasises the generally impaired transmural access of endothelium-dependent vasorelaxants to reach the endothelial cell layer.

In contrast to cannulated segments mounted in conventional pressure systems, the lumen of the mounted vessel in the impedance planimetry system might communicate with the organ bath through the cannulas as well as the small side branches left open. Therefore, the observed small relaxations of coronary arteries to higher concentrations of bradykinin are assumed to be the result of an eventual direct contact of the substances with the intimal layer. However, the small magnitude and the poor reproducibility of this result indicate

that access to the endothelium through these pathways is also markedly limited.

Endothelium-dependent relaxations are markedly affected by the level of agonist-induced tone (Dainty *et al*., 1990). Therefore, it was of interest to evaluate whether eversion influences the contractile properties of vascular smooth muscle. The results of the comparative investigations on the concentration-response relationship for K^+ and $PGF_{2\alpha}$ indicate that contractile responses of vascular smooth muscle in porcine coronary artery segments are independent of the vessel configuration. These findings, in accordance with those of Makujina *et al*. (1995) obtained on wire-mounted rings from porcine epicardial coronary arteries, suggest that everted arterial segments represent a suitable model for studying vascular smooth muscle function.

The difference between normal and everted segments with regard to endothelium-independent vascular responses concerned only an earlier onset and faster time-course when the agents were administered from the intimal as compared to the adventitial side. Since the effect of these substances is mediated by vascular smooth muscle, the differences in the time-course suggest that the diffusion barrier, at least in part, is located in the adventitial layer. Lovich & Edelman (1995) have demonstrated in rat abdominal aorta that the diffusive barrier imposed by the adventitia depends on its thickness. Our morphological investigation on porcine large coronary arteries have indicated that the adventitia is the dominant layer in these vessels and may occupy 40–60% of the vessel wall (Tankó *et al*., unpublished observations). This observation seems to be in accordance with the functional findings of the present study indicating the presence of a diffusion barrier imposed by the adventitia.

From the biomechanical point of view, it can be speculated that eversion may change the local wall stress experienced by the different components of the vessel wall in the everted as compared to the normal configuration. Therefore, the contribution of biomechanical factors to the differences seen in the time-course of the contractile and relaxant responses of coronary segments in the two different configurations cannot be excluded. However, as long as the constitutive law of vascular smooth muscle remains undetermined (Fung, 1981), only further biomechanical investigations can quantify these differences in wall stress distribution and determine their importance.

The pD_2 values for bradykinin obtained by impedance planimetry in everted preparations contracted with $PGF_{2\alpha}$ are comparable with previously reported values obtained in wire-mounted porcine coronary artery rings (e.g. Xu *et al*., 1996; Nagao & Vanhoutte, 1992). In addition, the vascular sensitivity and maximal relaxant responses to cumulatively administered bradykinin show no significant differences when compared under isobaric and isometric conditions within the same $PGF_{2\alpha}$-contracted coronary segment, indicating that the mode of recording vascular responses has no significant influence on the endothelium-dependent relaxant properties of the vessels.

Until now the study of endothelium-dependent responses with the impedance planimetry technique has been hampered by the lack of effect of endothelium-dependent vasodilators. The present study indicates that eversion of the preparation allows the investigation of these responses. Eversion of the segment by itself has no advantage, but it opens for two experimental approaches which are not possible with the wire-based (isometric) and conventional (isobaric) pressure techniques. Firstly, the investigation of the effect of axial stretch on the pharmacodynamics of large coronary arteries under *in vitro* conditions (Frøbert *et al*., 1996). This is relevant for these vessels as they experience not only circumferential but also axial extensions *in vivo* induced by changes in blood pressure and gross movement of the heart during the cardiac cycle (Osol, 1995). Second, it is possible to study endothelium-dependent responses in the same vascular segment, as illustrated in Figure 4, under both isometric and isobaric conditions. Although it is a disadvantage that eversion changes the normal vessel configuration, it allows the investigation of the effect of axial stretch and comparison of endothelium-dependent responses in cylindrical segments from large coronary arteries under isometric and isobaric conditions.

In conclusion, the reduced endothelium-dependent responses to bradykinin found in porcine large coronary arteries studied by the impedance planimetry technique seem to be due to the presence of an effective diffusion barrier in the thick wall of these preparations, while enzymatic degradation, luminal pressure, and contractile responses do not appear to play a major role. Furthermore, the impedance planimetry technique applied to everted cylindrical segments offers a new experimental approach for the study of endothelial and vascular smooth muscle function in medium-sized arteries under both isometric and isobaric conditions.

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