Coronary denervation attenuates coronary constriction induced by muscarinic receptor stimulation in pigs

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Abstract

Objectives: We tested the hypothesis that coronary denervation attenuates the reactivity of the coronary vessel to cholinergic stimulation. Methods: Heart rate, left ventricular (LV) pressure, LV dP/dt, coronary blood flow at the left anterior descending (LAD) coronary artery, and epicardial ECG mapping were measured before and after topical application of 1% methacholine to the LAD in 10 pigs anesthetized with alpha-chloralose (100 mg/kg, i.v.); these were compared with 10 other pigs submitted 2 weeks previously to a denervation of the LAD with phenol. Coronary denervation was confirmed in all cases by adrenergic histofluorescence and by acetyl-cholinesterase staining. Isolated LAD rings from 10 additional pigs (5 controls and 5 treated with phenol) were stimulated with endothelin-1 to verify whether phenol affected coronary reactivity to noncholinergic stimulation. Results: Methacholine induced a fall in coronary blood flow (10.3 ± 5.3 ml/min vs 4.8 ± 6.2 ml/min, ANOVA: P < 0.001), a drop in systolic LV pressure (113 ± 19 mmHg vs 93 ± 19 mmHg, P < 0.001) and LV dP/dt (1608 ± 363 mmHg/s vs 1203 ± 302 mmHg/s, P = 0.02) and elevation of the ST segment (1.4 ± 0.9 vs 11.1 ± 4.7 mV, P < 0.001) in controls. These changes were not preceded by heart rate variations and were inhibited by atropine. As compared to controls, phenol-treated pigs showed a smaller decline in coronary blood flow (13.1 ± 4.5 ml/min to 10.4 ± 5.4 ml/min, P < 0.001), a lower drop in LV pressure (107 ± 20 mmHg to 100 ± 19.7 mmHg, P < 0.001) and lesser ST segment elevation (2.2 ± 1.7 mV to 5.6 ± 4.2 mV, P < 0.001). Isolated LAD rings contracted after exposure to endothelin-1 in both controls and phenol-treated pigs (3.5 ± 0.7 g vs 2.4 ± 1.0 g, P = 0.06). Conclusions: Coronary denervation attenuates coronary constriction induced selectively by direct muscarinic receptor stimulation in the in situ pig heart.

Keywords: Coronary denervation; Myocardial ischemia; Pig, anesthetized; Autonomic nervous system; Coronary artery tone

1. Introduction

Clinical and experimental studies have demonstrated that coronary vasospasm can be either precipitated or attenuated by maneuvers that alter the cardiac autonomic nervous tone [1,2]. Acetylcholine or methacholine are able to induce coronary constriction in patients with Prinzmetal's form of variant angina [3,4], in tranquilized baboons [5], in isolated, donor-perfused rat hearts [6], in isolated coronary arteries of pigs [7], and in perfused murine hearts submitted to increased release of acetylcholine during cardiac vagal nerve stimulation [8]. By contrast, prevention of coronary spasm has been achieved after administration of atropine [3] or after surgical cardiac sympathetic denervation (pexectomy) in patients with Prinzmetal's angina [9]. Theoretically, selective denervation of the coronary artery may protect the vessel against induction of cholinergic coronary constriction, although this possibility has not yet been proven.

In this study we have tested the hypothesis that selective coronary denervation attenuates coronary vasoconstriction. Specifically, we have examined vascular reactiv-

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ity after coronary denervation with phenol in a new model of in vivo selective methacholine-induced coronary spasm in pigs.

2. Methods

2.1. Experimental preparation

Twenty White Large pigs (30–35 kg) of either sex underwent an aseptic left lateral thoracotomy at the 5th intercostal space under general anesthesia with thiopental sodium (30 mg/kg, i.v.) and mechanical ventilation. The rib was removed and the pericardium was opened. In 10 cases the left anterior descending (LAD) coronary artery was dissected at its origin along a length of 5 mm and a small gauze soaked in an 88% aqueous solution of carbolic acid (phenol) was applied three times to the pericoronary region. Epicardial spreading of phenol was avoided by drying the margins of the treated area. Topical application of phenol is expected to disrupt the coronary nerves because this procedure caused denervation of the distal myocardium in pigs [10]. In the remaining 10 pigs the LAD was not dissected and phenol was not applied (sham-operated controls). The thorax was closed and pleural air was aspirated. The animals were allowed to recover and received analgesia and antibiotic therapy.

Two weeks after the operation all 20 pigs were submitted to a midsternotomy under metamidate anesthesia (4 mg/kg, i.v.) followed by alpha-chloralose (100 mg/kg, i.v.). Pulmonary ventilation was maintained with a pressure respirator. The pericardium was opened and its free margins were sutured to the borders of the sternotomy to cradle the heart. The LAD was dissected both 20 mm below its origin for later application of methacholine and 5 mm distal to this site for coronary blood flow measurements. Left ventricular (LV) pressure was sampled with a pig-tail 7F catheter introduced percutaneously into the right femoral artery. Pressures were measured with a Hewlett Packard 7754B recorder and the LV dP/dt was derived from the LV pressure signal with a Hewlett Packard 8814A derivative computer. Arterial blood gases were measured at regular intervals and were kept within normal limits. Blood losses were compensated by intravenous infusion of isotonic saline solution through a peripheral abdominal vein.

Pigs were handled in accordance with the Guide for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1985). This study was approved by the Committee on Ethics of our Institution.

2.1.1. Epicardial mapping. Extracellular DC electrograms were recorded at the anterior surface of the left ventricle [11] using 32 electrodes sutured to a rubber membrane (3 parallel rows with 5 mm interelectrode space). The membrane was oriented parallel to the LAD and was gently sutured to the epicardium with Prolene 5/0 snare. The membrane covered epicardial areas proximal and distal to the site of methacholine application. Electrodes were polyethylene tubes of 0.5 mm diameter containing a cotton thread filled with isotonic saline solution. A silver chloride interface connected the electrodes with a 32-channel differential amplifier system. The 0 mV potential reference electrode was placed at the mediastinal fat. Samples of 2 s duration were digitized at 500 Hz and stored in a computer. In addition, selected analog electrograms were recorded with a 7-channel Elema ink jet polygraph. In each potential we measured the ST segment shift at a constant interval (120–150 ms) after the beginning of the QRS complex [11].

2.1.1.2. Coronary blood flow. Systolic, diastolic, and mean coronary blood flow were measured with a Skalar MDL 1401 electromagnetic recorder. The flowmeter probe (1.5 or 2.0 mm ID) was placed at the LAD 5–10 mm below the site of methacholine application avoiding compression of the coronary vessel. The study protocol began when baseline mean coronary blood varied less than 10% during a period of 15 min.

2.1.1.3. Coronary cholinergic constriction. Vasoconstriction of the LAD was induced by local application of methacholine. The LAD was dissected 2 cm distal to its origin along a length of 5 mm and a small (5 mm2) gauze soaked in a 1% aqueous solution of methacholine was applied to this adventitial area for 15 s. To avoid spreading of methacholine over the epicardium the gauze was slightly compressed before application to release excess liquid.

2.1.1.4. Muscarinic receptor blockade. The inhibitory effects of atropine on methacholine action were assessed in 5 out of the 10 control pigs. Thirty minutes after the first methacholine test these 5 pigs received atropine (0.04 mg/kg, i.v.) and a second methacholine test was performed 2 min thereafter.

2.1.1.5. Neuroanatomical studies. Coronary innervation was evaluated by glyoxylic-induced adrenergic histofluorescence [12] and by acetylcholinesterase reaction [13] in transverse block sections containing the coronary vessel, the perivascular adipose tissue, and a small portion of the underlying myocardium. Blocks were taken at the proximal segment of the LAD and right coronary artery and were immediately frozen with liquid nitrogen. In phenoltreated pigs the LAD samples were obtained at the site of phenol application and 2 cm distal to this region.

Sections of 20 to 30 μm were cut in a −20°C cryostat and were immersed in glyoxylic acid (glyoxylic acid Sigma, 2 g; glucose, 5.4 g; sodium phosphate, 5.5 g; distilled water, 100 ml; 10 N sodium hydroxide to titrate the solution to pH 7.4; and distilled water to reach a final
volume of 150 ml) for 15 s. Thereafter, preparations were consecutively incubated at 37°C for 45 min and at 85°C for 10 min. They were immediately observed in a Leitz fluorescent microscope with a K 490 filter.

Histochemical demonstration of acetylcholinesterase activity was performed with the "direct-coloring" copper ferrocyanide method [13]. Samples were preincubated for 15 min in ice-cold 0.1 M phosphate buffer (pH 7.0) in the presence of 10% formaldehyde. Incubation was carried out at 37°C for 1.5 to 3.5 h using acetylthiocholine iodide as the substrate. Qualitative myocardial fiber density relative to normal tissue was determined at ×100 magnification by two observers.

2.2. Protocol

Heart rate, CBF, LV pressure, LV dP/dt, conventional ECG, and epicardial mapping were recorded at baseline and every 15 s after application of methacholine for a period of 5 min. During the ensuing 5 min samples were obtained at 60 s intervals. At the end of the experiment the heart was removed for anatomical analysis.

2.3. In vitro studies

To support the concept that attenuation of cholinergic coronary constriction by phenol is linked to denervation rather than to a nonspecific vascular side effect of this drug, we analyzed the response of phenol-treated coronary arteries to noncholinergic stimulation with endothelin-1 (Sigma Quimica S.A.). Endothelin-1 was tested in vitro because in vivo pericoronary application of a gauze soaked in a 0.005% solution of this drug for a period of 2 min, failed to induce significant coronary or hemodynamic changes in 5 out of the 10 control pigs. Therefore, the proximal segment of the LAD was removed under general barbiturate anesthesia in 10 additional pigs (5 controls and 5 treated with phenol). The arterial tissue was placed in Krebs bicarbonate solution containing (mmol/l): NaCl 123, KCl 5, CaCl₂ 1.6, MgCl₂ 1.2, NaHCO₃ 25, CaNa₂ EDTA 0.026, glucose 11.1. In pigs treated with phenol we studied the LAD segment just distal to the site of phenol exposure. The LAD was cut in rings of 2 to 3 mm long leaving the endothelium intact; these were mounted in organ baths containing the Krebs solution (pH = 7.4, osmolarity = 300 mOs/l) aerated with 95% O₂ and 5% CO₂ thermostatically maintained at 37°C. The arterial segments were attached to an isometric force transducer Letica TRI 201 (Barcelona, Spain) and changes in tension were recorded by a Nihon Kohden RTA-1200 thermal array polygraph connected on line. Resting tension was adjusted to 2 g and tissues were allowed to stabilize for 1 h before they were stretched to their optimal length of contraction. Tension responses were tested after addition of 0.5 μM endothelin-1 to the incubation medium.

2.4. Data analysis

Data are expressed as mean ± s.d. Differences between groups were evaluated by repeated measures analysis of variance using the SYSTAT Inc. statistical software. The statistical significance of within-subject changes was assessed by the linear polynomial contrast derived from repeated measurements. Differences in the time course

![Fig. 1](image-url)  
**Fig. 1.** Effects of adventitial application of methacholine to the left anterior descending (LAD) coronary artery on ECG (lead II), epicardial electrogram at the LV anterior region (Epi), phasic and mean coronary blood flow (CBF), LV pressure (LVP), and LV dP/dt in an open-chest pig of the control group. Numbers indicate the mean CBF values. Methacholine elicited a marked fall in CBF, LVP, and LV dP/dt associated with ST segment elevation. Recovery of coronary blood flow gave rise to a hyperemic reaction (5 min).
between groups were assessed by comparing the linear polynomial contrast between them (interaction term). If these differences were significant, within-subject changes were assessed in each group by the linear polynomial contrast, whereas pooled contrast was applied when the differences were not significant. The simple sequentially rejective Holm’s method [14] was used to adjust for multiple comparisons. Only those with a $P$ value < 0.005 were considered significant.

3. Results

3.1. Effects of methacholine

As illustrated in Fig. 1 and Table 1, control pigs submitted to pericoronary application of methacholine developed a drop in coronary blood flow ($P < 0.001$), a fall in systolic LV pressure ($P < 0.001$) and LV $dP/dt$ ($P = 0.002$), and an elevation of the ST segment ($P < 0.001$) at the surface of the anterior LV region (Fig. 2). These alterations were notpreceded by changes in heart rate ($105 \pm 16$ beats/min at baseline vs $104 \pm 16$ beats/min at the onset of coronary blood flow drop, $P = NS$). The drop in coronary blood flow was maximal 1 to 3 min after methacholine treatment and fully recovered during the ensuing 5 to 8 min giving rise to an hyperemic reaction (Fig. 1). The total coronary blood flow deprivation induced by methacholine, calculated as the area under the curve of coronary blood flow reduction, was $36 \pm 22$ ml/min $\times$ min in the 10 pigs of the control group. A second methacholine test performed 2 h later in 5 out of the 10 control pigs induced comparable coronary blood flow and hemodynamic changes (Table 2). By contrast, muscarinic blockade with atropine in the remaining 5 control pigs completely inhibited the hemodynamic and coronary blood flow ef-

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**Table 1**
Maximal effects of methacholine in 10 control pigs and in 10 pigs with coronary denervation induced by phenol 2 weeks before the study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Maximal change</th>
<th>$\Delta$ Change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$105 \pm 16$</td>
<td>$107 \pm 16$</td>
<td>$1 \pm 2$</td>
</tr>
<tr>
<td>Phenol</td>
<td>$118 \pm 21$</td>
<td>$122 \pm 19$</td>
<td>$3 \pm 4$</td>
</tr>
<tr>
<td>Mean CBF at the LAD (ml/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$10.3 \pm 5.3$</td>
<td>$4.8 \pm 6.2$</td>
<td>$-6.0 \pm 1.6$</td>
</tr>
<tr>
<td>Phenol</td>
<td>$10.4 \pm 5.4$</td>
<td>$10.4 \pm 5.4$</td>
<td>$-7.6 \pm 1.5$</td>
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<tr>
<td>LV systolic pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$113 \pm 19$</td>
<td>$93 \pm 19$</td>
<td>$-20 \pm 8.4$</td>
</tr>
<tr>
<td>Phenol</td>
<td>$107 \pm 20$</td>
<td>$100 \pm 19$</td>
<td>$-7 \pm 2.3$</td>
</tr>
<tr>
<td>LV $dP/dt$ (mmHg/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>$1608 \pm 363$</td>
<td>$1203 \pm 302$</td>
<td>$-404 \pm 203$</td>
</tr>
<tr>
<td>Phenol</td>
<td>$1458 \pm 529$</td>
<td>$1291 \pm 540$</td>
<td>$-167 \pm 104$</td>
</tr>
<tr>
<td>ST segment (mV)</td>
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<td></td>
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<tr>
<td>Control</td>
<td>$1.4 \pm 0.9$</td>
<td>$11.4 \pm 4.7$</td>
<td>$9.7 \pm 4.7$</td>
</tr>
<tr>
<td>Phenol</td>
<td>$2.2 \pm 1.7$</td>
<td>$5.6 \pm 4.2$</td>
<td>$2.4 \pm 2.1$</td>
</tr>
</tbody>
</table>

**Table 2**
Comparative effects of two methacholine tests spaced 30 min apart in 5 open-chest pigs

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Maximal change</th>
<th>$\Delta$ Change from baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st MCH</td>
<td>$97 \pm 16$</td>
<td>$99 \pm 13$</td>
<td>$2 \pm 3$</td>
</tr>
<tr>
<td>2nd MCH</td>
<td>$92 \pm 15$</td>
<td>$94 \pm 14$</td>
<td>$2 \pm 1$</td>
</tr>
<tr>
<td>Mean CBF at the LAD (ml/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st MCH</td>
<td>$9.0 \pm 2.0$</td>
<td>$7.8 \pm 3.0$</td>
<td>$-2.2 \pm 1.8$</td>
</tr>
<tr>
<td>2nd MCH</td>
<td>$8.6 \pm 1.9$</td>
<td>$3.4 \pm 2.3$</td>
<td>$-5.2 \pm 2.1$</td>
</tr>
<tr>
<td>LV systolic pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st MCH</td>
<td>$113 \pm 11$</td>
<td>$89 \pm 12$</td>
<td>$-23 \pm 4.4$</td>
</tr>
<tr>
<td>2nd MCH</td>
<td>$107 \pm 10$</td>
<td>$88 \pm 12$</td>
<td>$-20 \pm 7.0$</td>
</tr>
<tr>
<td>LV $dP/dt$ (mmHg/s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st MCH</td>
<td>$1513 \pm 357$</td>
<td>$1148 \pm 339$</td>
<td>$-365 \pm 138$</td>
</tr>
<tr>
<td>2nd MCH</td>
<td>$1456 \pm 274$</td>
<td>$1159 \pm 391$</td>
<td>$-297 \pm 162$</td>
</tr>
</tbody>
</table>

Abbreviations: MCH = methacholine; CBF = coronary blood flow; LAD = left anterior descending coronary artery; LV = left ventricular. See text for statistical analysis.
effects of methacholine. Phenol-treated pigs submitted to adventitial application of methacholine showed (Fig. 3, Fig. 4, and Table 1) a lower fall in coronary blood flow (P < 0.001), lower drop in LV pressure (P < 0.001), and lower ST segment elevation (P < 0.001) than controls. Total coronary blood flow deprivation was also less marked (10 ± 13 ml/min x min, P = 0.009).

3.2. Effects of endothelin-1

In vivo pericoronary application of endothelin-1 in 5 controls 30 min after recovery from previous methacholine failed to induce significant changes in coronary blood flow (9.6 ± 4.6 ml/min to 9.0 ± 4.1 ml/min), LV pressure (115 ± 11 mmHg to 112 ± 7 mmHg), and LV dP/dt (1591 ± 241 mmHg/s to 1563 ± 317 mmHg/s).

Fourteen LAD coronary rings from 5 control pigs and 18 rings from 5 phenol-treated pigs constricted after incubation with endothelin-1. Constriction began 5 to 15 s after drug exposure and reached a maximal plateau during the ensuing 3 to 5 min. Maximal developed tension was 3.5 ± 0.7 g in control rings and 2.4 ± 1.0 g in phenol-treated rings (P = 0.06).

3.3. Autonomic innervation

Control pigs showed a rich network of coronary cholinergic and adrenergic nerve fibers immersed in the perivascular adipose tissue. These fibers course parallel to the coronary artery and do not penetrate beyond its adventitial-medial border (Fig. 5A and Fig. 6A). By contrast, phenol-treated pigs showed a marked loss of adrenergic and cholinergic pericoronary nerve fibers (Fig. 5B and Fig. 6B). Myocardial samples from the anteropapical region show an abundant cholinergic and adrenergic innervation in control pigs but, in contrast, they showed a loss of autonomic fibers in phenol-treated animals. The LAD at the site of phenol application showed a nonspecific perivascular inflammatory reaction with preservation of the arterial wall structure (Fig. 7).

4. Discussion

4.1. Cholinergic vasoconstriction in normal and denervated coronary arteries

The major observation in this study was that pericoronary denervation attenuates the reactivity of the coronary artery to adventitial cholinergic stimulation with methacholine. Pigs submitted 2 weeks earlier to a pericoronary application of phenol show a loss of cholinergic and adrenergic pericoronary nerve fibers below the site of phenol exposure and, as compared to controls, phenol-treated pigs develop a less marked decline in coronary blood flow and a lower drop in LV pressure and LV dP/dt after adventitial administration of methacholine.

Pericoronary application of methacholine in control pigs induces a marked fall in regional coronary blood flow, a depression in LV function, and local ST segment elevation. The drop in coronary blood flow is caused by coronary vasoconstriction at the site of methacholine exposure,
Fig. 4. Graphic illustration of the sequential changes in heart rate, coronary blood flow, and ST segment induced by adventitial application of methacholine in 10 controls and in 10 phenol-treated pigs. Symbols represent the mean value, and bars one standard deviation.

as we have previously documented angiographically in 6 pigs (unpublished data). In agreement with this observation, other studies have shown that coronary smooth muscle strips constrict after incubation with cholinergic agents in pigs [15,16], in guinea pigs [17], and in humans [18,19].

By contrast, the response to systemic administration of cholinergic drugs varies among species [1] due to differences in endothelial relaxing receptors [20,21]. For example, acetylcholine elicits coronary dilation in dogs [22,23], coronary constriction in pigs [16,24,25], and dose-dependent constriction in baboons [5]. The vasoconstrictor effect of methacholine is likely exerted via the coronary muscarinic receptors because atropine inhibited the hemodynamic effects of methacholine. In fact, in vitro studies in swine confirm that muscarinic receptors, predominantly located on vascular smooth muscle cells, mediate cholinergic coronary constriction [15].

The mechanism by which coronary arteries treated with phenol respond less vigorously to methacholine is unknown. Our study suggests that this protective effect is related to cholinergic coronary denervation induced by phenol because the vascular effects of methacholine are mediated by muscarinic receptors. Hypothetically, coronary denervation might alter the number and/or the function of coronary muscarinic receptors thus leading to a depressed vascular reactivity to cholinergic stimuli. Although the effects of coronary denervation on local muscarinic receptors are unknown, indirect information from studies on murine brain models shows that dopamine denervation [26] or neural lesioning [27] induce downregulation or decreases in muscarinic receptor density.

It is unlikely that attenuation of cholinergic coronary constriction in phenol-treated arteries resulted from a toxic drug effect rather than from denervation. Indeed, we observed no structural damage of coronary vessel at the site of phenol exposure and, furthermore, rings of phenol-treated LAD contract after incubation with endothelin-1 indicating, indirectly, that coronary smooth muscle cells are functionally preserved [28,29]. The putative effect of topical phenol to disrupt neural fibers has been recognized in other studies [10,30].

The concurrent sympathetic coronary denervation induced by phenol might modulate the coronary vasoconstriction elicited by methacholine. However, adrenoceptor blockade does not prevent the vascular response to acetylcholine in some species [5].

4.2. Considerations on the model

Extraluminal application of methacholine allows the assessment of coronary reactivity at preselected vascular

Fig. 5. Glyoxylic-induced adrenergic histofluorescence of a proximal segment of the left anterior descending (LAD) coronary artery in a control pig (A) and in a phenol-treated pig (B). Adrenergic fibers were observed in the adventitial-medial region of the vessel in the normal pig, whereas these fibers were absent in the pig treated with phenol. Magnification ×40 in (A) and ×200 in (B).

Fig. 6. Acetylcholinesterase reaction of a proximal segment of the left anterior descending (LAD) coronary artery in a normal pig (A) and in a pig treated with phenol (B). The figure shows a positive cholinesterase reaction at the adventitial region (arrow) and disappearance of cholinergic fibers after phenol exposure. Magnification ×100. L = coronary lumen.

Fig. 7. Hematoxylin-eosin staining of a frozen proximal segment of the left anterior descending (LAD) coronary artery submitted 2 weeks earlier to phenol application. Phenol induces a nonspecific perivascular inflammatory reaction (arrow) with preservation of the arterial wall structure. Magnification ×100. L = coronary lumen, A = adventitial medial border.
levels and avoids systemic drug effects. Analysis of coronary vasomotion at predetermined sites may be relevant because coronary reactivity is greater in distal than in proximal segments of the LAD in pigs and dogs [17,31,32] and on the other hand, coronary adrenoceptors are not uniformly distributed in pigs [33]. The lack of bradycardia and hypotension preceding the changes in coronary blood flow induced by extraluminal methacholine suggests that coronary constriction is not triggered by a reflex sympathetic stimulation.

In this study we purposely avoided direct manipulation of the endothelium since the integrity of this vascular structure determines the response of coronary artery to systemic acetylcholine in humans [34]. However, endothelial cells do not play a major role in cholinergic spasm in pigs [16].

4.3. Clinical implications

This study provides insight into the potential role of coronary denervation in preventing cholinergic coronary vasospasm. The present data support previous observations made in a limited series of patients with Prinzmetal's angina who showed reduction in the number of anginal attacks after surgical cardiac plexectomy [9]. Selective coronary denervation like that in this study would be more advantageous than cardiac plexectomy because the former is expected to cause less extensive cardiac denervation. However, further studies are required not only to assess the potential clinical applicability of coronary denervation but also to rule out the possibility that the protective effect of denervation vanished over time. In addition, further studies should verify whether a denervated coronary artery may develop hypersensitivity to circulating catecholamines.

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