Reactivity of Isolated Porcine Coronary Resistance Arteries to Cholinergic and Adrenergic Drugs and Transmural Pressure Changes

Kengo Nakayama, George Osol, and William Halpern

The reactivity of porcine intramyocardial resistance arteries (223 ± 7 μm i.d., n = 30) was investigated with a pressurized in vitro preparation. Diameter changes in response to acetylcholine and to adrenergic drugs and dynamic changes in transmural pressure changes were investigated. Acetylcholine produced concentration-dependent constrictions, causing maximal reductions of 71 ± 3% in lumen diameter, with EC₅₀ values averaging 1.9 × 10⁻⁷ M (n = 7). These responses were inhibited by atropine (10⁻⁷ M) and therefore were mediated by muscarinic receptors. In addition, acetylcholine did not elicit relaxation in nine out of 10 vessels precontracted with U46619 (10⁻⁷ M). Norepinephrine and epinephrine never produced constrictions (n = 6) even in the presence of propranolol (10⁻⁶ M). Both norepinephrine and isoproterenol caused dose-dependent relaxations in acetylcholine-precontracted vessels, with IC₅₀ values of 8.2 × 10⁻⁷ M (n = 5) and 6.6 × 10⁻⁷ M (n = 6), respectively. These relaxations were suppressed by propranolol. Between transmural pressures of 10 and 90 mm Hg, there was no intrinsic myogenic tone (n = 7). In addition, the vessels responded only passively to sudden pressure changes of 40 mm Hg. In all vessels, the functional integrity of the endothelium was verified by relaxations to substance P (10⁻⁷ M) and/or bradykinin (10⁻⁸ M). This is the first in vitro study of coronary resistance arteries that demonstrates that 1) acetylcholine is a potent constrictor of these arteries, suggesting that parasympathetic mechanisms may play an important role in coronary blood flow regulation; 2) α-adrenergic influence is minimal or entirely absent; and 3) these arteries do not possess an intrinsic myogenic tone and respond passively to transmural pressure change. Hence, myogenic mechanisms do not appear to be of primary importance in the autoregulation of coronary blood flow. (Circulation Research 1988;62:741–748)

Clinical evidence suggests that parasympathetic mechanisms may play an important role in the regulation of coronary blood flow and vasospasm. For example, administration of parasympathomimetic agents such as methacholine and pilocarpine induced attacks in patients with variant angina, which could be suppressed by atropine.¹ ² Tamada et al.³ also observed parasympathetic hyperactivity in the patients with vasospastic angina. Previous experimental studies of cholinergic mechanisms were mainly performed on dogs, in which vagal stimulation increases coronary blood flow.⁴ It is becoming apparent, however, that these results cannot be generalized to other mammals and to humans without verification because acetylcholine (ACh) mainly elicits constrictions in isolated, large epicardial arteries obtained from humans, pigs, and sheep.⁵ ⁶ In contrast, relaxation is the predominant response of dog and monkey arteries.⁵ ⁶ ⁸ From the viewpoints of both size and pharmacological reactivity, porcine coronary arteries appear to be more similar to those of humans than to the canine arteries.

In addition to species variation, there is also evidence for significant differences in vascular reactivity occurring as a function of vessel size.⁵ ⁶ ¹⁰ Most studies have been concerned with adrenergic and cholinergic responses of large epicardial arteries, although it is generally acknowledged that the major component of blood flow resistance and, hence, control of blood flow resides in smaller arteries and arterioles.

The major objective of the present study was to investigate and characterize the reactivity of porcine intramyocardial resistance coronary arteries to cholinergic and adrenergic drugs that, to our knowledge, have not been previously studied. The reasons these vessels are considered to be true resistance arteries are detailed in the text.

The contribution of myogenic mechanisms to coronary blood flow autoregulation has been suggested,¹¹ ¹² but this phenomenon is difficult to evaluate under in vivo conditions since metabolic and neurogenic influences are difficult to exclude. By using an in vitro technique in which arteries are cannulated and pressurized,¹³ we also measured the diameter responses of isolated vessels as a function of transmural pressure. Previous work on rat and calf cerebral arteries¹⁴ ¹⁵ confirmed that this approach, the use of pressurized cylindrical segments, is effective in quantitating both the level of intrinsic tone and diameter adjustments that favor autoregulation of blood flow.
Materials and Methods

Vessel Preparations

Hearts from male and female pigs (n = 30) with body weights of 100–150 kg were obtained from local slaughterhouses within 2 minutes of death and immediately immersed in cold oxygenated physiological saline solution (PSS). Intramyocardial arteries 3–4 mm in length were carefully dissected from branches of the left anterior descending artery. The dissection technique involved keeping the tissue in the dissecting dish filled with cold oxygenated PSS, locating a branch 4–6 cm from the coronary ostium, and following this branch into the right ventricular myocardium until an appropriate arterial segment was obtained. Within 2 hours of death, excised arteries were cannulated and tied to inlet and outlet polyethylene tubes filled with PSS (150 μm o.d.) within a specially designed arteriograph (Living Systems Instrumentation, Burlington, Vermont). Any small branches were tied with a strand of nylon suture. Oxygenated PSS was then flushed through the artery to remove residual blood.

The solution in the vessel chamber of the arteriograph was circulated continuously from an external reservoir of PSS that was maintained at a temperature of 36° to 37° C and vigorously bubbled with 95% O2-5% CO2 gas. Under these conditions, pH, Po2, and Pco2 values were between 7.38 and 7.45, 450 and 550 mm Hg, and 32 and 36 mm Hg, respectively. The arteriograph also contained a gassing stone within the vessel bath, and a milled channel surrounding the bath filled with circulating warm water permitted accurate control of temperature. Therefore, the conditions described above were maintained within the bath even when PSS circulation was stopped during pharmacological studies. The vessels were initially pressurized to 20 mm Hg and equilibrated for 1–2 hours without intraluminal flow.

The arteriograph was placed on the stage of a microscope that was coupled to a TV camera. Lumen diameter, wall thickness, and transmural pressure were measured simultaneously with a video-electronic dimension analyzer13 (Living Systems Instrumentation) and a pressure transducer placed proximal to the vessel.

The vessels were classified into four groups, as shown in Table 1, and described in the following sections of text.

Reactivity to Pharmacological Drugs

The responses of small coronary arteries to all pharmacological agents were examined at a steady transmural pressure of 40 mm Hg. Every drug was added directly and cumulatively to the vessel chamber with the exception of those vessels that were preincubated with blocking agents. After exposure to an agonist, the vessels were washed with PSS for at least 20 minutes before another application of drugs. During administration of drugs, circulation of solution through the vessel chamber was stopped, but the temperature, pH, Po2, and Pco2 of bath solution were kept constant as described above. Antagonists were added to the circulating solution at least 30 minutes before the addition of agonists.

ACH contractile responses in the presence and absence of atropine (Group A, n = 7). ACh was applied in cumulative doses until no further constrictions were obtained. ACh was again added cumulatively to the vessels preincubated with atropine (10−7 M). Substance P (10−8 M), an endothelium-dependent dilator of the porcine coronary artery,16 was applied to the vessels precontracted with ACh (4 × 10−7 M), followed by papaverine (10−4 M).

Maximal responses to ACh were expressed as percentage reductions in lumen diameter. The EC50 values (concentration of ACh causing 50% of maximal response) were calculated for each vessel with linear regression analysis. Responses were normalized to the maximal response measured in each vessel to ACh in the absence of atropine.

Responses to epinephrine, norepinephrine, and isoproterenol in the presence and absence of propranolol (Group B, n = 6). Epinephrine (E) and norepinephrine (NE) were applied to the vessels in a concentration range of 10−7 to 6 × 10−4 M. Afterward, the vessels were precontracted with ACh (4 × 10−7 M) to investigate relaxation responses to isoproterenol (ISO) and NE. ISO or NE was added cumulatively until no further relaxations were obtained. Vessels were then maximally relaxed with papaverine (10−4 M). Substance P (10−8 M) was applied to the vessels precontracted with ACh (4 × 10−7 M), followed by papaverine (10−4 M). This series was repeated in the vessels preincubated with propranolol (10−6).

Diameter responses to ISO and NE were expressed as a percentage of the maximal relaxation induced by papaverine. The IC50 values (concentration of each agonist causing 50% of maximal relaxation) were calculated as described above for the EC50 calculations. The maximal relaxation of each vessel to ISO and NE was taken as 100%, and relaxation at each concentration is expressed as a percentage of this value to obtain concentration-response curves of ISO and NE in the presence and absence of propranolol.

Verification of endothelial function (Group C, n = 10). The relaxation responses to ACh, substance P, and bradykinin were investigated in the vessels precontracted with U46619 (10−7 M), a stable thromboxane A2 (TXA2) analogue. ACh was added cumulatively beginning with the concentration of 10−9 M until a response was recorded. Substance P (10−8 M) and

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<th>Table 1. Experimental Protocols</th>
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<td>Group</td>
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<td>Total</td>
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ACh, acetylcholine; NE, norepinephrine; E, epinephrine; ISO, isoproterenol.
bradykinin (10⁻⁸ M), endothelium-dependent dilators of porcine coronary arteries, were applied to U46619-precontracted vessels, followed by papaverine (10⁻⁴ M) to provide an index of relaxation capability. Afterward, six of the 10 vessels were preincubated for 30 minutes with methylene blue (10⁻⁵ M), an inhibitor of guanylate cyclase. Then, substance P and bradykinin (10⁻⁸ and 3 × 10⁻⁸ M) relaxations were investigated in TXA₂-precontracted vessels, followed by papaverine (10⁻⁴ M).

An earlier study in our laboratory described a technique for endothelial removal in small (200-µm) mesenteric arteries, which involves perfusion with CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate), a nonionic, nonenaturing detergent. Therefore, in the remaining four vessels, 0.5% CHAPS was intraluminally perfused for 90 seconds to remove the endothelium, followed by 30 minutes perfusion with prewarmed and oxygenated PSS. Relaxation responses to ACh, substance P, bradykinin, and papaverine were examined once more, as described above.

Reactivity to Transmural Pressure Changes (Group D, n = 7)

After equilibration, transmural pressure was cycled twice from 10 to 90 mm Hg, with each cycle taking about 8 minutes. The axial length of each vessel was set at a pressure of 60 mm Hg by just removing any buckling of the segment with a nonrotating head micrometer attached to the inlet cannula. After this cycle, vessels were kept at 10 mm Hg and then subjected to a series of 10-mm Hg pressure steps up to 90 mm Hg for the investigations of distensibility and myogenic properties. For both increasing and decreasing steps, pressure was maintained until a stable lumen diameter was obtained. After two or three repetitions, the amplitude of the pressure steps was changed to 40 mm Hg to examine the myogenic response to sudden large changes in pressure. Afterward, this series was repeated in the presence of papaverine (10⁻⁴ M) to obtain the passive pressure-diameter characteristics. All pressure steps were performed without intraluminal flow and under continuous circulation of the bath solution.

At the conclusion of an experiment, the presence of the endothelium was functionally tested by relaxation responses to substance P (10⁻⁸ M) in the vessels precontracted with ACh (4 × 10⁻⁷ M).

Morphological Examinations

After experimentation, five vessels were immersed in 10% formalin for routine light microscopic examination at 40 mm Hg. To verify the presence of the endothelium, five vessels were immersed in 2.5% glutaraldehyde in Millonig’s phosphate buffer for 30–60 minutes and were stored in buffer solution for scanning electron microscopic examination. To examine endothelial cell denudation and morphological changes of smooth muscle cells by CHAPS, two of four vessels perfused with CHAPS were prepared for transmission electron microscopic examination. In three other vessels, after completing the experimental protocol, en face silver staining was also used to assist in examining the endothelial surface.

Solutions and Drugs

The PSS solution contained the following composition (mM): NaCl 119, NaHCO₃, 24, KCl 4.7, KH₂PO₄, 1.18, MgSO₄-7H₂O 1.17, CaCl₂ 1.6, glucose 5.5, and EDTA 0.026.

The following drugs were used in this study: acetylcholine chloride, (-)-norepinephrine bitartrate, (-)-epinephrine bitartrate, DL-isoproterenol HCl, DL-propranolol HCl, atropine sulfate, papaverine HCl, substance P, bradykinin, methylene blue, CHAPS (Sigma Chemical, St. Louis, Missouri), and U46619 (Upjohn, Kalamazoo, Michigan).

Statistics

The results, shown in the text, tables, and figures, are presented as mean ± SEM (n = number of vessels). EC₅₀ and IC₅₀ values are expressed as the geometric mean ± SEM, determined from logarithmic values. Differences in agonist-induced responses with and without antagonists were tested with Student’s paired and two tailed t tests. Significance was considered at p ≤ 0.05.

Results

The lumen diameter and wall thickness of the small intramyocardial vessels used in the experiment were 223 ± 7 µm (150–285 µm; n = 30) and 37 ± 3 µm, respectively, at a transmural pressure of 40 mm Hg. Light microscopy of transverse sections revealed a well-developed media, which contained three to five layers of smooth muscle cells.

Responses to Acetylcholine

ACh (3 × 10⁻⁸ to 6 × 10⁻⁶ M) always produced a dose-dependent contraction in the resting vessels, causing maximal lumen diameter reductions of 71 ± 3%, with an EC₅₀ for ACh of 1.9 × 10⁻⁷ M. Atropine (10⁻⁷ M) significantly attenuated maximal responses of ACh (p < 0.01) (Figure 1) and produced a marked shift of ACh concentration-response curves to the right, as evidenced by an EC₅₀ of 5.5 × 10⁻³ M, which was about 300 times greater than that of controls.

ACh relaxation responses were examined in 10 U46619-precontracted vessels that were confirmed to relax to both substance P and bradykinin. As shown in Figure 2, in nine out of 10 vessels, ACh produced further constrictions of precontracted vessels, with a threshold of 3.6 ± 0.7 × 10⁻⁸ M. In only one vessel, ACh elicited relaxation that was inhibited and inverted to constriction after CHAPS treatment.

Responses to Epinephrine, Norepinephrine, and Isoproterenol

NE and E (10⁻⁷ to 6 × 10⁻⁴ M) did not induce contractions in either resting or precontracted vessels.
(n = 6) in both the absence and the presence of propranolol.

For the examination of relaxation responses to ISO and NE, vessels were precontracted with 4 x 10^-7 M ACh, which caused about 55% reduction in lumen diameter. One vessel was eliminated because of a technical error during the experiment. Results are summarized in Table 2 and Figures 3 and 4.

ISO (10^-4 to 10^-6 M) and NE (6 x 10^-8 to 3 x 10^-5 M) invariably produced concentration-dependent relaxations. Maximal relaxations to ISO and NE relative to those of papaverine were 97% and 86%, respectively. Corresponding IC50 values were 6.6 x 10^-8 and 8.2 x 10^-7 M (Figures 3 and 4). Propranolol (10^-6 M) did not affect the magnitude of ACh-induced precontraction (Table 2), but it significantly attenuated the maximal relaxations induced by ISO and NE. In the presence of propranolol, the IC50 of ISO and NE were two orders in magnitude greater than those of controls (Figures 3 and 4).

Functional and Morphological Verification of Endothelium

As shown in Table 3, all ACh-precontracted vessels (n = 20) examined in groups A, B, and D of this study relaxed to substance P (10^-8 M), which elicited 85% of the relaxation to papaverine.

As shown in Table 3, substance P and bradykinin (10^-8 M) produced large relaxations of vessels precontracted with U46619 (n = 10). These relaxations were completely inhibited after perfusion with CHAPS (Figure 2) and by methylene blue.

Scanning electron microscopy revealed that most endothelial cells were preserved even though there was minimal dropout. En face silver staining clearly outlined the borders of the endothelial cells. Transmission electron micrographs after CHAPS perfusion showed that the endothelial cells were not seen, that internal elastic lamina remained intact, and that the smooth muscle cells appeared to be intact.

Reactivity to Transmural Pressure Changes

During stepwise increases in transmural pressure from 10 to 90 mm Hg, average lumen diameter and wall thickness changed from 209 ± 14 to 257 ± 19 μm, and from 41 ± 4 to 36 ± 3 μm, respectively, in PSS. When vessels were preincubated in papaverine (10^-4 M), the lumen diameter changed from 215 ± 16 to 260 ± 19 μm. As shown in Figure 5, diameter at 10 mm Hg was approximately 80% of that at 90 mm Hg. The pressure-diameter relations with and without papaverine were not statistically different, indicating that these vessels did not possess an intrinsic myogenic tone. The vessels consistently followed this pressure-diameter curve, even when subjected to sudden pressure changes of 40 mm Hg.

Discussion

This is the first study to examine the pharmacological and mechanical reactivity of intramyocardial coronary resistance arteries with pressurized cylindrical segments. The major findings are 1) ACh is a potent constrictor of small intramyocardial arteries that have a functional and morphologically intact endothelium; 2) α-adrenergic receptors are not operative in these arteries; 3) these arteries do not possess an intrinsic tone and do not display myogenic responses to sudden pressure changes, at least under these in vitro test conditions.

We conclude that the small intramyocardial arteries examined in this study qualify as being true resistance arteries in the porcine coronary circulation for the following reasons. One must consider the large difference in heart sizes between pigs (300-400 g) and cats or rabbits (less than 20 g) used in previous investigations. Nellis et al.,20 using an electromechanical micromanipulator, found about a 40% decline from mean systemic pressure in 140-μm diameter arteries of the rabbit heart. Chilian et al.,21 using a similar technique, subsequently demonstrated that approximately 25% and 45% of the total coronary vascular resistance in the beating coronary vasculature of the cat resides proximal to prearterioles of 200 and 100 μm diameter, respectively. Comparably sized arteries of the rat, however, showed only minimal and inconsistent pressure differences from the ascending aorta.22

The lumen diameter of the arteries used in the present study averaged about 200 μm, which is 15–20 times smaller than the porcine conductance vessels.23 However, arteries of 100–200 μm, which are recognized as

![Figure 1. Concentration-response curves to ACh in the absence and presence of atropine (n = 7). Mean values: ●, control vessels; ○, vessels treated with atropine. Vertical bars, SEM. Atropine decreased maximal contraction to 89 ± 3% of controls (p<0.01). The EC50 (●) was 1.9 (1.5–2.5) x 10^-8 and 5.5 (4.7–6.5) x 10^-8 M in the absence and presence of atropine, respectively.](http://circres.ahajournals.org/cover)
resistance vessels in the cat and rabbit coronary circulations by the above studies, are only five times smaller than the conductance arteries of these species. Considerations of flow resistance based on Poiseuille's fourth power diameter relation and differential responsiveness of similar-sized arteries between the rat and cat or rabbit both predict that porcine small arteries could contribute at least as much and probably more to flow resistance as the small arteries of the cat or rabbit when related to the major epicardial arteries.

**Responses to Acetylcholine**

In contrast to several reports concerning large coronary arteries, very little is known about ACh responses of resistance-sized vessels. The observation by Knight et al. that intracoronary injection of ACh reduces coronary blood flow in the baboon independently of changes in myocardial metabolic demand supports our finding that ACh is a very powerful constrictor of intramyocardial resistance arteries. This contractile response is inhibited by atropine, suggesting that the effect is probably mediated by muscarinic receptors. In addition, the endothelium-dependent relaxation to ACh normally found in other arteries appears to be absent in these vessels.

In the last several years, many experiments have suggested that endothelial damage attenuates relaxation responses to ACh in large coronary arteries. Although there is no controversy regarding endothelium-dependent relaxation responses to ACh in canine or monkey coronary arteries, there is conflicting evidence concerning this response in human coronary arteries. For example, Förstermann et al. reported ACh relaxation responses in only three of 32 isolated coronary artery preparations from recipient hearts of transplant patients with cardiomyopathy. Other in vivo angiographic, as well as in vitro studies, support a constrictor effect of ACh on human large coronary arteries in the presence of the endothelium. Bossaller et al., on the other hand, demonstrated endothelium-dependent relaxations to ACh in similar preparations. There is also a report by Ludmer et al. using angiographic evaluation that shows relaxation response to ACh.

**FIGURE 2.** Typical lumen diameter traces for functional verification of the endothelium before and after CHAPS perfusion. Panel A: Vessel was precontracted with U46619 (10^{-7} M). ACh was applied in a cumulative manner beginning with 10^{-8} M. ACh concentration of 3 x 10^{-4} M produced further constriction in this vessel. SP (10^{-6} M) elicited almost complete relaxation. This vessel also relaxed to BRK (10^{-6} M). Panel B: After CHAPS perfusion, a similar protocol was repeated in the same artery. Again, the 3 x 10^{-4} M dose of ACh induced further constriction. Note the absence of SP and BRK (10^{-4} and 3 x 10^{-4} M) relaxations, however, and full relaxation to Pap (10^{-6} M). ACh, acetylcholine; SP, substance P; BRK, bradykinin; Pap, papaverine; W, wash. Large arrows, initial application of each drug; small arrows, cumulative applications of drug after initial dose.

**FIGURE 3.** Concentration-response curves to isoproterenol in the absence and presence of propranolol (n = 6). Mean values: Control vessels; o, vessels treated with propranolol. Vertical bars, SEM. Propranolol decreased maximal relaxation to 91 ± 3% of controls (p<0.05), and it shifted the concentration-response curve two orders of magnitude to the right, as evidenced by a change in the IC_{50} (Δ) from 6.6 (4.9-9.0) x 10^{-4} to 1.9 (1.6-2.2) x 10^{-5} M.
In this study, endothelial injury was minimized with cannulated cylindrical arterial segments in which the central portion of the segment was untouched. The effectiveness of this technique in preserving the endothelium was confirmed morphologically by scanning electron microscopy and silver staining. Furthermore, substance P or bradykinin, endothelium-dependent dilators of porcine coronary arteries, in vitro invariably produced relaxations of all vessels precontracted with ACh or a stable TXA₂ analogue. These relaxations were attenuated by methylene blue, a functional inhibitor of the action of endothelium-derived relaxing factor, and by removing the endothelial cells with CHAPS. For this combination of reasons, we conclude that it is unlikely that ACh constrictions of small intramyocardial arteries occurred as a result of endothelial damage.

One must consider whether the route of drug application might affect ACh responses of the vessels since Cohen et al. observed that large canine coronary arteries did not relax to ACh if applied only extraluminally, whereas these arteries did relax to intraluminally applied ACh. Earlier studies in our laboratory, however, demonstrated that rat mesenteric and cerebral arteries having the same order of magnitude of wall thickness as the vessels used in this study (20–40 μm) readily relax to superfused ACh. For this reason, the route of application in small vessels does not appear to be important, and ACh was applied only extraluminally.

In summary, we examined the ACh reactivity of porcine intramyocardial resistance arteries that may directly contribute to blood flow resistance and, hence, coronary blood flow regulation. ACh is a potent constrictor of these vessels, raising the possibility that parasympathetic mechanisms may play an important role in coronary blood flow regulation.

Responses to Epinephrine, Norepinephrine, and Isoproterenol

The responses of small coronary arteries to E, NE, and ISO suggest that 1) relaxation to NE and ISO may be mediated by β-adrenoreceptors because propranolol suppressed these relaxations; and 2) α-adrenoreceptors do not operate under in vitro conditions because of the absence of contraction to both NE and E, even in the presence of propranolol. These results confirm previous reports on the reactivity of small coronary arteries to adrenergic agonists in pigs and dogs. Again, species variations do exist: small coronary arteries from monkeys and humans contract slightly to NE and E in the presence of propranolol, but not in its absence, indicating the presence of some α-adrenoreceptors. Nevertheless, at this time, the consensus seems to be that β-adrenoreceptors are predominant and that α-adrenoreceptors are practically absent in small coronary arteries of most animals.

Reactivity to Transmural Pressure Changes

Autoregulation of coronary blood flow has been intensely studied with in vivo techniques and in

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**FIGURE 4.** Concentration-response curves to norepinephrine in the absence and presence of propranolol (n = 5). Mean values: •, control vessels; ○, vessels treated with propranolol. Vertical bars, SEM. Propranolol inhibited maximal relaxation to 75 ± 4% of controls (p < 0.01), and it shifted the concentration-response curve to the right, as evidenced by a change in the IC₅₀ (Δ) from 8.2 (6.2–11.0) × 10⁻⁷ to 9.4 (7.5–11.7) × 10⁻⁷ M.

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**TABLE 2.** Isoproterenol and Norepinephrine Relaxations in Arteries Precontracted With Acetylcholine (4 × 10⁻⁷ M)

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<thead>
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<th>Condition</th>
<th>n</th>
<th>ACh-precontraction (%)</th>
<th>Relaxation (%)</th>
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<tr>
<td><strong>Isoproterenol</strong></td>
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<td></td>
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</tr>
<tr>
<td>Without</td>
<td>6</td>
<td>54 ± 2</td>
<td>97 ± 2</td>
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<tr>
<td>With</td>
<td>6</td>
<td>58 ± 2</td>
<td>88 ± 3*</td>
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<tr>
<td><strong>Norepinephrine</strong></td>
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<tr>
<td>Propranolol</td>
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<td></td>
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<tr>
<td>Without</td>
<td>5</td>
<td>57 ± 7</td>
<td>86 ± 4</td>
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<tr>
<td>With</td>
<td>5</td>
<td>56 ± 4</td>
<td>64 ± 3†</td>
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</table>

* *p < 0.05, †p < 0.01 between propranolol without and with. NS, no significant difference between propranolol without and with.

ACh, acetylcholine.

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**TABLE 3.** Substance P and Bradykinin Relaxations in Arteries Precontracted With Acetylcholine (4 × 10⁻⁷ M) and U46619 (10⁻⁷ M)

<table>
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<th>Condition</th>
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<th>Precontraction (%)</th>
<th>Relaxation (%)</th>
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<tr>
<td><strong>Substance P</strong></td>
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<tr>
<td>(10⁻⁸ M)</td>
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<tr>
<td>ACh-precontraction</td>
<td>20</td>
<td>53 ± 4</td>
<td>85 ± 3</td>
</tr>
<tr>
<td>U46619-precontraction</td>
<td>10</td>
<td>38 ± 4</td>
<td>77 ± 6</td>
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<tr>
<td><strong>Bradykinin</strong></td>
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<tr>
<td>(10⁻⁸ M)</td>
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<tr>
<td>U46619-precontraction</td>
<td>10</td>
<td>37 ± 4</td>
<td>82 ± 7</td>
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</table>
perfused hearts. However, little is known about the contribution of myogenic mechanisms in resistance-sized arteries in controlling coronary blood flow since metabolic mechanisms cannot be excluded under the condition of in vivo and perfused heart. Our experimental system practically eliminates metabolic influences, thereby facilitating the direct study of myogenic responses to transmural pressure changes in a physiological range.

The results show that intramyocardial arteries do not possess intrinsic tone and do not develop active responses to sudden changes in transmural pressure. In contrast to these results, several investigators observed basal tone and/or phasic activity of isolated coronary conductance arteries from humans, pigs, and rats.30-32

The possibility of impairment of spontaneous tone of vessels during the process of our dissection and mounting could be a factor. However, our procedures for vessel preparation are similar to those used by others, and the dissection and mounting techniques for 100-200-μm vessels are well established in our laboratory.13,14,18 Cox18 demonstrated the advantage of the technique of using pressurized cylindrical segments for the mechanical vessel studies compared with using the ring or strip segment preparation. Also, cylindrical preparations decrease the likelihood of damaging the vessel because the central portion of the segment was untouched.

Duling et al documented that isolated arterioles become reactive to ACh, NE, and adenosine as skill of dissection improves. Our vessels had very consistent pharmacological reactivity to ACh, NE, and other drugs including adenosine (data not shown); additionally, the endothelium, which may affect the vascular tone, was well preserved both functionally and morphologically. Furthermore, we consistently observe myogenic tone and diameter responses to pressure that favor autoregulation in similarly sized rat cerebral arteries with the same experimental setup and protocols as in the present study,5 which supports the effectiveness of our in vitro technique for quantitating myogenic properties of small arteries. Therefore, these considerations make it unlikely that the absence of myogenic tone and responses to transmural pressure changes are due to experimental difficulties.

Although myogenic mechanisms have been shown to contribute to autoregulation of blood flow in most vascular beds,14,15 there are no studies explicitly demonstrating the existence of myogenicity in the coronary circulation. In some studies,11,12,15 even brief coronary occlusion was followed by a reactive hyperemia. The authors suggested the involvement of metabolic mechanisms in the regulation of coronary flow because such brief occlusion may not cause the release of myocardial metabolites. However, Schwartz et al found that only a single ventricular extra-activation, causing a brief and transient increase in cardiac metabolic demand, is followed immediately by a compensatory coronary vasodilation, indicating minimal responsibility of myogenic mechanisms for coronary autoregulation. In summary, at this time, most in vivo investigations demonstrate that metabolic mechanisms were mainly responsible for coronary autoregulation, supporting our results of minimal involvement of myogenic mechanisms in coronary blood flow regulation, at least in arteries of about 200 μm diameter.

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93–103

**KEY WORDS**: coronary circulation • coronary vessels • acetylcholine • adrenergic drugs • myogenic mechanism
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