Contractile Responses of Canine Coronary Collateral Vessels

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SUMMARY

This study examines the responses of coronary collateral vessels to various vasoconstrictors and compares them with responses of 3 different divisions of the native coronary arterial trees in dogs. Only high concentrations of phenylephrine induced contractions in collateral vessels and medium coronary arteries, whereas even a high concentration of this agent did not affect small coronary arteries. Lower concentrations of serotonin or ergonovine elicited contractions in collateral vessels, and medium and large coronary arteries, but even a high concentration of these agents did not affect small coronary arteries. Angiotensin II (10^{-7} M) elicited prominent contractions in the following order: small vessels, collateral vessels, medium and large coronary arteries. These results indicate that well-developed collateral vessels behave like native medium diameter coronary artery, and that the tonus of collateral vessels is possibly influenced by various vasoactive substances.

Additional Indexing Words:

Large coronary artery	Medium coronary artery	Small coronary artery
Phenylephrine	Serotonin	Ergonovine
Angiotensin II		

IT has been repeatedly shown that the gradual coronary artery occlusion results in the development of collateral vessels which protect the jeopardized myocardium from necrosis.¹⁾⁻³⁾ Several reports have suggested that collaterals grow actively rather than expand passively when the surrounding cardiac muscle is made ischemic. The kinetics of this vascular growth and transformation process in response to myocardial ischemia have been studied in detail.⁴⁾⁻⁶⁾ Furthermore, morphological studies have shown that the normal heart has small interconnecting anastomotic vessels which are non-func-

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tioning, although there is much variation between species and some variation within a species. Following coronary occlusion, the dilatation of these communicating vessels can be demonstrated in the early stage and, subsequently, an active proliferative growth process involving both intimal and medial layers occurs in the later stage. It makes these vessels indistinguishable from native coronary artery.^{4),7)}

It is speculated that coronary collateral flow may be augmented by various vasodilators in animals and humans and that well-developed collateral vessels possess an active vasomotor tone.⁸⁾⁻¹²⁾ However, the contractile properties of collateral vessels in response to various vasoactive substances have not been studied.

This study was undertaken to examine whether isolated helical strips of coronary collaterals are responsive to different vasoconstrictors under the controlled *in vitro* condition. In addition, the contractile characteristics of these vessels were compared with those of native coronary arterial trees.

METHODS

(1) Ten adult mongrel dogs of either sex, weighing 10 to 15 Kg, were anesthetized with sodium pentobarbital (25 mg/Kg i.v.) and ventilated with a respirator. Then the chest was opened in the 4th left intercostal space under aseptic conditions and the pericardium was cut. The proximal portion of the left circumflex artery was dissected from the surrounding connective tissues. An ameroid constrictor was positioned around it without any knowledge concerning the preexisting intercoronary collateral development in each dog. The pericardium was incompletely closed with a silk suture and the chest was closed. The animals received antibiotics (i.m.) for 3 post-operative days.

Two to 4 months after the implantation, the animals were reanesthetized with sodium pentobarbital (25 mg/Kg i.v.) and sacrificed by exsanguination. The heart was promptly resected and put into oxygenated Krebs-Henseleit solution of the following composition (mM); NaCl, 118; KCl, 4.0; CaCl₂, 1.5; MgSO₄, 1.2; NaH₂PO₄, 1.2; NaHCO₃, 25 and glucose 5. The well-developed epicardial collateral vessels were macroscopically distinguished from the poorly developed ones which were excluded from the following experiment. These vessels were subdivided into 3 portions, i.e., the donor, mid and recipient portions. Each portion of these vessels was removed and cut into helical strips, approximately 0.5 mm wide and 10 mm long. The strips were suspended in 30-ml muscle chambers containing Krebs-Henseleit solution for the recording of isometric force. The buffer was equilibrated at 37°C with a 95% O₂ and 5% CO₂ gas mixture and the final pH was approximately 7.4.

An initial preload of 500 mg was applied to each strip. After 2 hours of equilibration, a test contraction was induced by raising the KCl concentration of the buffer to 24 mM. When the developed tension attained its peak value, the strips were relaxed by rinsing them with standard buffer. Concentration-response relations for phenylephrine, serotonin and ergonovine were determined by the cumulative addition of each drug to the bath fluid. Since angiotensin II showed a prominent tachyphylaxis, the concentration-response relations could not be obtained. Therefore we evaluated their responses to 10^{-7} M angiotensin II. After each experiment, we reevaluated the evoked contractions by raising the KCl concentration to 24 mM, confirming that no changes in vascular reactivity had occurred during the experimental period.

To assess the morphological characteristics of collateral vessels, the vascular samples used for mechanical studies were fixed in 10% formaldehyde solution. The specimens were stained with hematoxylin-eosin or van Gieson elastica and examined microscopically.

(2) In another series of experiments, we evaluated the vascular reactivity to vasoactive substances in the different regions of native canine coronary arteries. Fifteen adult mongrel dogs were anesthetized with sodium pentobarbital (25 mg/Kg i.v.) and sacrificed by exsanguination. The different divisions of coronary arterial trees (Large, 1–1.5 mm outer diameter [O.D.]; Medium, 0.5–1 mm O.D.; Small, less than 0.5 mm O.D.) were dissected and cut into helical strips (Large, 1.5 mm wide and 20 mm long; Medium, 1 mm wide and 10 mm long; Small, 0.5 mm wide and 10 mm long). As described previously, the strips were suspended in muscle chambers. An initial preload of 1,500 mg, 1,000 mg, and 500 mg was applied to large, medium, and small coronary arteries, respectively. After 2 hours of equilibration, we examined the responses of the arteries to the same vasoactive substances as used in experiment (1).

All results were expressed as mean $\pm$ standard error of the mean. The significance was assessed by Student's t-test for unpaired samples.

RESULTS

(1) Histological study

Two to 4 months after an ameroid implantation, 8 dogs had macroscopically well-developed epicardial collateral vessels. The other 2 dogs had poor collateral vessels and were excluded from the further study. Histological examination of the specimens prepared from the mid portions of epicardial collateral vessels revealed the same structural changes as reported by Schaper.^{4), 13)} The prominent features were the increased diameter, wall thick-

Fig. 1. Concentration-response relations for phenylephrine in large, medium, and small coronary arteries. The contractile responses to phenylephrine are expressed as a percentage of their maximal responses.

Table I. Effect of Various Agonists on the Large, Medium, and Small Coronary Arteries from Mongrel Dogs

	Large C.A.	Medium C.A.	Small C.A.
Ang II/KCl	0.626 ± 0.117 (n=11)	1.038 ± 0.146 (n=8)	1.280 ± 0.538 (n=6)
Phenyl./KCl	0.786 ± 0.095 (n=11)	0.037 ± 0.010 (n=10)	0 (n=6)
Erg./KCl	0.649 ± 0.101 (n=11)	0.327 ± 0.046 (n=10)	0 (n=6)
5HT/KCl	0.713 ± 0.236 (n=7)	0.101 ± 0.022 (n=5)	0 (n=2)

Values are mean $\pm$ SE.

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$, **** $p < 0.001$.

KCl 20 mM, Ang II 10^{-7} M, Phenylephrine 10^{-4} M, Ergonovine 10^{-5} M, 5HT 10^{-5} M.

ness, fragmentation of the internal elastic membrane, widening of sub-endothelial space and several layers of circular smooth muscle cells in the tunica media.

(2) Responses of large, medium, and small coronary arteries to vasoactive substances

The log concentration-response relations for phenylephrine are shown in

Fig. 2. Concentration-response relations for serotonin in large, medium, and small coronary arteries.

Fig. 3. Concentration-response relations for ergonovine in large, medium, and small coronary arteries.

Fig. 1. Phenylephrine effectively contracted large coronary arteries. The threshold concentration and ED_{50} value were 10^{-9} M and 10^{-6} M, respectively. The medium coronary arteries responded to phenylephrine only at a high concentration (10^{-4} M). Phenylephrine failed to contract small coronary arteries. As shown in Table I, the maximal response ratio of phenylephrine to 20 mM KCl was 0.79 ± 0.10 in large, 0.04 ± 0.01 in medium, and 0 in small coronary arteries.

The log concentration-response relations for serotonin (5HT) are shown in Fig. 2. Serotonin in lower concentrations could induce prominent contractions both in large and medium coronary arteries. The threshold con-

Fig. 4. Concentration-response relations for phenylephrine in 3 segments of coronary collateral vessels.

Table II. Effect of Various Agonists on Collateral Vessels

	Donor	Mid	Recipient
Ang II/KCl	1.668 ± 0.397 (n=6)	1.193 ± 0.249 (n=6)	1.131 ± 0.221 (n=6)
Phenyl./KCl	0.122 ± 0.047 (n=6)	0.038 ± 0.011 (n=6)	0.076 ± 0.017 (n=6)
Erg./KCl	0.385 ± 0.141 (n=6)	0.159 ± 0.036 (n=6)	0.287 ± 0.078 (n=6)
5HT/KCl	0.276 ± 0.085 (n=6)	0.106 ± 0.022 (n=6)	0.276 ± 0.145 (n=6)

Values are mean $\pm$ SE.

KCl 20 mM, Ang II 10^{-7} M, Phenylephrine 10^{-4} M, Ergonovine 10^{-5} M, 5HT 10^{-5} M.

centration and ED_{50} value in both large and medium coronary arteries were 10^{-10} M and 10^{-8} M, respectively. These response curves for serotonin in the two divisions are very similar. But the maximal response ratio of serotonin to 20 mM KCl was 0.71 ± 0.24 in large, and 0.10 ± 0.02 in medium coronary arteries (Table I). Even a high concentration of serotonin could not elicit any contractions in small coronary arteries.

Fig. 3 depicts the log concentration-response relations for ergonovine. Ergonovine is an ergot alkaloid which is routinely used to provoke coronary vasospasm in patients with angina pectoris. Ergonovine in lower concentrations could induce prominent contractions in both large and medium coronary arteries. The threshold concentration and ED_{50} value in both large and medium coronary arteries were 10^{-10} M and 10^{-8} M, respectively. These response curves for ergonovine in the two divisions of coronary arteries were

Fig. 5. Concentration-response relations for serotonin in 3 segments of coronary collateral vessels.

very similar. However, the maximal response ratio of ergonovine to 20 mM KCl was 0.65 ± 0.10 in large and 0.33 ± 0.05 in medium coronary arteries (Table I). No concentration of ergonovine up to 10^{-5} M elicited contractions in small coronary arteries.

It is known that angiotensin II shows tachyphylaxis. We compared the response of large, medium, and small coronary arteries to 10^{-7} M angiotensin II. The response ratio of angiotensin II to 20 mM KCl was 0.63 ± 0.12 in large, 1.04 ± 0.15 in medium, and 1.28 ± 0.54 in small coronary arteries. This suggests that angiotensin II receptors are more dominant in small than large coronary arteries.

(3) Responses of collateral vessels to vasoactive substances

We macroscopically divided epicardial collateral vessels into 3 parts, i.e., donor, mid, and recipient parts. The outer diameter of each part was about 0.5 mm, i.e., the same as medium coronary arteries. Fig. 4 shows the log concentration-response relations for phenylephrine in each part. The response curves for phenylephrine in the 3 divisions of coronary collateral vessels were identical. Only the high concentration of phenylephrine (10^{-4} M) could elicit contractions in the 3 parts. The maximal response ratio of phenylephrine to 20 mM KCl was 0.12 ± 0.05 in the donor, 0.04 ± 0.01 in the mid, and 0.08 ± 0.02 in the recipient part of collateral vessels (Table II). This shows that phenylephrine caused the strongest contractions in the donor part and the weakest in the mid-part of collateral vessels. The value in the mid-part of collateral vessels is similar to that in medium diameter (0.5–1 mm) native coronary arteries (Tables I, II).

The log concentration-response curves for serotonin are depicted in Fig.

Fig. 6. Concentration-response relations for ergonovine in 3 segments of coronary collateral vessels.

5. In each part of collateral vessels, even a low concentration of serotonin could elicit contractions. The threshold concentration and ED_{50} value in all parts of collateral vessels were 10^{-9} M and 3×10^{-8} M, respectively. The maximal response ratio of serotonin to 20 mM KCl was 0.28 ± 0.09 in the donor, 0.11 ± 0.02 in the mid, and 0.28 ± 0.15 in the recipient part of collateral vessels (Table II). The value in the mid-part of collateral vessels is similar to that in medium diameter native coronary arteries (Tables I, II).

Fig. 6 shows the log concentration-response curves for ergonovine. A comparatively low concentration of ergonovine could elicit contractions in all 3 parts. The maximal response ratio of ergonovine to 20 mM KCl was 0.39 ± 0.14 in the donor, 0.16 ± 0.04 in the mid, and 0.29 ± 0.08 in the recipient part of collateral vessels (Table II). The value in the mid-part of collateral vessels is similar to that of medium diameter (0.5–1 mm) native coronary arteries (Tables I, II).

We compared the responses of these 3 parts to 10^{-7} M angiotensin II. The response ratios for angiotensin II to 20 mM KCl were 1.67 ± 0.40 in the donor, 1.19 ± 0.25 in the mid, and 1.13 ± 0.22 in the recipient part of collateral vessels. The value from mid collateral vessels is compatible with that of medium sized native coronary arteries (Tables I, II).

DISCUSSION

Postmortem examinations have documented the existence of coronary interarterial anastomoses in the normal heart.^{14,15)} These collateral connections increase in size and number following coronary occlusion.

Schaper et al have reported that collaterals grow actively rather than expand passively when the surrounding cardiac muscle is made ischemic.⁶⁾ DNA synthetic activity in coronary collateral vessels was assessed with [³H] thymidine in dogs subjected to progressive stenosis of the left circumflex coronary artery for different periods of time. Proliferative activity was highest at the level of the smallest diameters of the collateral vessels (mid zone). Dense labelling was detected in the intima and media.⁶⁾

Histological studies have postulated that small interconnecting anastomotic vessels have been transformed into overstretched arterioles with a dilated lumen and with little or no muscular coat in the early stages after coronary artery occlusion. In later stage, however, the collaterals actively grow, and possess relatively small lumens with thick muscular walls containing several layers of smooth muscle cells. Our histological studies confirmed that the features of well-developed collateral vessels resemble those of arteries rather than arterioles.^{4),18)}

It has been suggested that several vasodilators, including nitroglycerin, dipyridamole, prostacyclin, and adenosine, may increase blood supply into the ischemic myocardium through collateral channels.^{8)~10),12)} It is difficult to decide whether coronary collateral vessels actively dilate in response to these drugs. These observations must be interpreted carefully by considering separately the direct and the indirect effects of these drugs on the coronary circulation in intact animals. An increase in blood flow into collateral-dependent myocardium could arise from vasomotor changes in the collaterals as well as vasodilation in the donor coronary artery and the vascular bed which the collaterals supply. It is controversial whether well-developed collateral vessels behave primarily as passive, connecting channels or whether they acquire vascular reactivity. In order to clarify the contractile properties of collateral vessels, we have examined the effect of vasoactive agents on arterial strips of collateral vessels. The comparative observations were made between collateral vessels and sections of the native coronary artery.

The present data suggest that the well-developed collateral vessels gain pharmacological receptors. This implies that coronary collateral blood flow may be influenced by active changes in the vasomotor tone of well-developed collateral vessels.

We extended the scope of the study to examine the pharmacological heterogeneity of different parts of the coronary vessels. Phenylephrine failed to elicit any contractions of small coronary arteries, whereas this agent elicited concentration-dependent contractions of large coronary arteries. Phenylephrine produces arterial contractions by the activation of the α_1 adrenergic receptor, but this agent also activates β -adrenoceptors in higher concentra-

tions. Our preliminary data show that phenylephrine activates β -adrenoceptors at concentrations greater than 10^{-5} M, and the activation of β -adrenoceptors induces relaxation of canine coronary arteries. This observation confirms previous reports that α_1 adrenergic receptors are located preferentially in the proximal portion of coronary arterial trees.¹⁶⁾ Serotonin is a potent vasoconstrictor of large and medium sized coronary arteries,¹⁷⁾ but it did not elicit any contractions of small coronary arteries. Porquet et al reported previously that serotonin produced contractile responses in the proximal but not in the distal segment of the coronary artery under *in vitro* conditions. Our result confirms her observations.¹⁸⁾ Ergonovine was also a potent vasoconstrictor of large and medium coronary arteries. We have reported that ergonovine induces coronary contractions by stimulation of serotonin receptors of vascular smooth muscle.¹⁷⁾ Angiotensin II, 10^{-7} M, elicited prominent contractions of all parts of the coronary arteries, and it induced the most prominent contractions in small coronary arteries.

These results suggest that different vasoactive agents have a different potency spectrum of vasoconstrictor activity in different vascular beds. In addition, well-developed collateral vessels acquire pharmacological receptors and respond to various vasoconstrictor stimuli. The contractile properties of well-developed collateral vessels resemble those of medium diameter coronary arteries.

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