Endothelial cells regulate cardiac contractility

(endothelium/endothelial factors/cardiac energetics/cardiac autoregulation)

CLAUDIO RAMACIOTTI*, ANGELA SHARKEY*, GEORGE MCCLELLAN†, AND SAUL WINEGRAD‡‡

1Department of Physiology, School of Medicine, University of Pennsylvania, and †Department of Pediatric Cardiology, Children's Hospital of Philadelphia, Philadelphia, PA 19104

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ABSTRACT Endothelial cells lining the lumen of blood vessels contain the receptors for many substances that alter the contractile tone of smooth muscle in the walls of the blood vessels. In response to their interaction with the signal substances, the endothelial cells release vasoactive factors that modify the contractile state of the vascular smooth muscle. This study was conducted to determine if endothelial cells can also modulate the contraction of cardiac muscle cells and contribute to the physiological regulation of the heart. The venous effluent from the coronary circulation of an isolated perfused working heart was reoxygenated and used to superfuse a trabecula isolated from the right ventricle of another heart. The peak tension and the duration of the contraction of the trabecula were reversibly altered by the effluent fluid. The change in the contraction of the trabecula during its exposure to coronary effluent was inhibited by selectively damaging the endothelial cells in the trabecula before the application of the coronary effluent. The magnitude and direction of the effect of the coronary venous effluent were sensitive to the metabolic and mechanical conditions under which the isolated perfused heart was contracting at the time the effluent was collected. These observations indicate that cardiac tissue can release a substance or substances into the coronary circulation that induce the production of cardioactive factors by endothelial cells.

In their classical study, Furchgott and Zawadski (1) demonstrated that endothelial cells lining the lumen of blood vessels play an obligatory role in the vasoactive effect of acetylcholine. The mechanism by which the endothelial cells exerted their influence was the release of a diffusible substance that interacted with the smooth muscle cells in the wall of the blood vessel (2). This substance, originally called endothelial-derived relaxing factor, has subsequently been identified as nitric oxide (3, 4). A second endothelial-derived factor that has very powerful vasoconstricting activity has been isolated and sequenced and named endothelin (5). Endothelin, a 21-residue peptide, has receptors on cardiac cells, and it has a powerful positive inotropic effect due to change in intracellular Ca and in the response of the contractile proteins to activator Ca (6, 7).

Damage of the endocardial endothelium in isolated bundles of cardiac muscle decreases the peak tension that is developed and induces an earlier onset of relaxation (8, 9). There is some indication that a factor released by cultured endothelial cells can reverse the effects of damaging the endocardium (10, 11). In other work quantitative histochemistry has been used to demonstrate that factors derived from coronary blood vessels can modulate the ATPase activity of myocardial actomyosin (12). The results have also shown that an as yet unidentified factor regulates the response of the contractile proteins to activator Ca by a mechanism that does not involve β-adrenergically mediated phosphorylation of the inhibitory subunit of troponin (13). This study has been conducted to examine whether endothelial-derived factors can modulate cardiac contractility and whether the as yet unidentified factor with cardioactive properties can come from endothelial cells.

METHODS

Thin trabeculae or papillary muscles were isolated from the endocardial surface of the right ventricle of hearts removed from male rats weighing between 200 and 300 g. Great care was taken during the dissection to avoid touching the surface of the tissue bundle with a dissection instrument. The tissue bundle was suspended between a Grass force transducer and a mechanical ground at ~3.2-μm sarcomere length and bathed in a Krebs solution (118 mM NaCl/4.8 mM KCI/1.0 mM KH2PO4/1.2 mM MgSO4/27 mM NaHCO3/2.5 mM CaCl2/11.1 mM glucose, pH 7.4) in which 95% O2/5% CO2 continuously bubbled. Temperature was maintained at 30 ± 0.1°C with a continuously flowing water jacket attached to a constant temperature circulating bath. The tissue was stimulated to contract with electric shocks delivered through punctate electrodes at 0.2 Hz. The stimuli were 5 msec in duration with a voltage 20% above the level necessary to produce maximum force response. Tension was continuously monitored with a strip chart recorder, and periodically the contractile wave form was captured by a computer through an A/D converter. Each wave form shown in the figures is a computer-averaged value of five consecutive contractions. The volume of the bathing solution was several hundred times the volume of the tissue. Replacement of one aliquot of Krebs solution with another had no effect on the contraction.

Isolated, perfused working hearts were prepared by a small modification (13) of the Neely–Morgan preparation (14) using hearts taken from male rats weighing between 250 and 350 g. The aorta, pulmonary artery, left atrium, and right atrium were cannulated to control left atrial filling pressure and to measure cardiac output, coronary sinus flow, total coronary flow, and systolic and diastolic pressure. The rate of contraction was maintained at ~250 beats per min. The perfusate was Krebs solution (same as used for isolated trabeculae) equilibrated with 95% O2/5% CO2 and maintained at 37°C. Arterial and venous oxygen tension were continuously monitored with oxygen electrodes in the left atrium and pulmonary artery. Cardiac power was calculated as mean pressure times cardiac output. Forty minutes of perfusion were allowed for recovery from dissection and stabilization. The total volume of fluid in the perfused heart apparatus was ~300 ml, and this was recirculated throughout the run. Coronary flow in the preparations that were studied averaged 21 ± 2 ml/min at a left atrial filling pressure of 10 cm of H2O and 25 ± 3 ml/min at a left atrial filling pressure of 15 cm of H2O. Therefore, the perfusion fluid passed through the coronary circulation the equivalent of four or five times per

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‡To whom reprint requests should be addressed.
hour. During the experiment left atrial filling pressure was maintained at 10 cm of H2O except for specific periods during which the filling pressure was increased to 15 cm of H2O.

At appropriate times after the contractile activity of an isolated trabecula taken from another heart had stabilized, the coronary sinus effluent was collected from the pulmonary artery for 1.5 min and reoxygenated with 95% O2/5% CO2. The effluent was brought to 30°C and used to replace the Krebs solution bathing the trabecula, which was also maintained at 30°C. The reoxygenated coronary sinus effluent was applied to the trabecula 3 min after collection. The change in the shape of the wave form of contraction was monitored continuously.

At least seven different preparations were studied for each protocol, with each preparation consisting of a trabecula and a perfused heart from different animals. Significance was evaluated by the Student's t test.

RESULTS

Isolated trabeculae were stimulated at 0.2 Hz for 90–150 min to allow the wave form of the contraction to stabilize following the dissection. During this period, peak tension declined by 16% ± 5% (n = 18) and the time to 50% relaxation decreased by 19% ± 3% (Fig. 1), but there was little, if any, change in the maximum rate of rise of tension. After contractility had stabilized, there was very little change in the strength or time course of the contraction for at least an additional 2 hr. Previous work has indicated that the stabilization process in isolated trabeculae may be related to the change in the environment of the endothelial cells. As a result of the termination of perfusion of the blood vessels and the superfusion of the bundle, shear force and oxygen tension are different in the isolated tissue from that in the in situ perfused heart (12).

The changes observed during the period of stabilization are qualitatively the same as those produced when the endocardial endothelium was markedly damaged and its function interrupted by a 1-sec exposure to 0.5% Triton X-100 in Krebs solution before the process of stabilization was complete (8, 9). The endocardium in thin trabeculae includes at least 85% of the total endothelium exclusive of the capillaries (data not shown). The remaining 15% is present in the small arteries and veins in the tissue. The change in the contraction in response to Triton X-100 required only 2–5 min. If contractility had been allowed to stabilize first, a brief exposure to detergent had very little effect on the contraction, reducing peak force development of isolated trabeculae by 6% ± 2% (n = 18) (Fig. 2). The size of the change was larger if stabilization was not complete at the time of the exposure to the detergent. The maximum rate of rise of tension was unaffected by the detergent. Damage to the endocardial endothelium was confirmed in a few instances by staining with 1% Evans blue dye (8, 9). Regardless of when detergent was briefly applied, contractility was very stable within 2–5 min. There were no indications of any direct alteration of the myocytes by the detergent, as judged by developed force, rate of rise of force, rate of relaxation, onset of relaxation, or ultrastructure.

From the similarity in the changes in the contraction during stabilization and following damage to the endocardial endothelium, it appears that during stabilization there is a marked diminution or disappearance of activity of the endocardial endothelium on the contraction of the myocellular cells. Either the endothelial cells are deteriorating or there is a combination of washout of substances stimulating the endothelium and decay of effects induced prior to the in vitro suspension of the tissue. Transmission electron micrographs indicate that the structure of the endocardial endothelial cells remains intact during the period of stabilization and argue against deterioration of endothelial cells.

To determine whether the influence of endothelial cells could play a physiological role in regulating the contraction of cardiac muscle, isolated working hearts were prepared and periodically coronary sinus effluent was collected, reoxygenated, and applied to the isolated trabecula.

During the period in which the trabecula was bathed with the coronary sinus effluent, the contraction changed (Fig. 3). The alteration in developed force produced by the effluent was not due to changes either in temperature, which was

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**Fig. 1.** Change in the wave form of an isolated trabecula during the period of stabilization following dissection. (A) Stabilization has been allowed to go to completion. There is a progressive decline in peak tension and a progressively earlier onset of relaxation with time until the wave form stabilizes. Stabilization has occurred by 90 min. (B) The trabecula was exposed to 0.5% Triton X-100 for 1 sec before stabilization was complete. Exposure to Triton X-100 produces the same change as stabilization. The times indicate time from the removal of the heart from the rat prior to isolation of the trabecula.

**Fig. 2.** Effect of treatment with 0.5% Triton X-100 in Krebs solution for 1 sec on the contraction. Each contraction is a computer average of five consecutive contractions. The contractility of the tissue was allowed to stabilize for 100 min before the application of the detergent, and the post-detergent record was taken 10 min after the removal of the detergent. There is no change in the wave form of the contraction. Following the very brief exposure to 0.5% Triton X-100, the contraction remains unchanged over a prolonged period of time.
maintained constant within 0.1°C, or in pH, which changed by <0.05 pH unit because of buffering. Replacing the Krebs solution bathing the muscle by another aliquot of Krebs solution, using the same procedure as was used with coronary sinus effluent, produced no significant changes in the contraction. The nature of the change correlated with the oxygen tension in the coronary sinus effluent at the time it was collected from the perfused heart, even though the content of oxygen had been raised to the original arterial value by reequilibration with 95% O2/5% CO2 before the fluid had been applied to the trabecula. Normally, in an unselected group of isolated perfused working hearts, there will be a range of coronary venous oxygen tensions. We have used this variability in testing the relation between oxygen tension and the properties of the coronary venous effluent on the contractility of isolated cardiac trabeculae. When coronary sinus oxygen tension was high, the effluent increased the force of contraction, and when oxygen tension was low, the effluent lowered the force of contraction of the trabecula (Fig. 4).

The change in the contraction began within 2 min and was complete by 10 min. The new contraction wave remained stable for an additional 5 min, the longest period of exposure totaling 15 min. When the coronary venous effluent was replaced by standard Krebs solution, the shape of the contraction returned to the control configuration within 5–10 min in most cases. In ≈25% of the trabeculae studied, the contraction had a lower peak force after exposure to the coronary venous effluent, even though the peak force may have been increased during exposure to the effluent. Exposure to a second aliquot of effluent collected under the same hemodynamic conditions produced a response in the same direction as the first aliquot (not shown).

A relation between oxygen tension and change in contractility produced by coronary venous effluent continued to exist when the work performed by the isolated heart producing the effluent was increased by 24% ± 5% as a result of raising left atrial filling pressure from 10 to 15 cm of H2O (Fig. 4). Increase in preload on the heart, however, increased the slope of the relation. This change is unlikely to be related to any change in the oxygen tension at the myocyte as a result of the increase in work performed. Coronary venous PO2 did not change significantly, and the increase in coronary flow...
was sufficient to account for the increment in oxygen uptake that was necessary. The efficiency with which the heart used oxygen in the performance of work, calculated as mean arterial pressure times flow/coronal flow times the arterio-venous difference in oxygen tension, increased by a small amount when the cardiac output rose in response to the elevated filling pressure. In looking for the cause of the change in slope, we found no relation between the change in force developed by the 14 trabeculae studied and cardiac output, work, or coronary flow in the isolated perfused hearts. A possible explanation is that stretch of the cells is responsible for the change in the effect of the coronary venous effluent on the contraction of the trabecula. There are, however, no rigorous data to prove this speculation.

Elimination of the function of the endothelial cells in the trabecula inhibited the effect of the coronary venous effluent on the contractility of a trabecula. After the first application of coronary venous effluent had altered contractility of a trabecula, the trabecula was treated briefly with 0.5% Triton X-100. A second aliquot of coronary venous effluent, collected from the same perfused heart under the same mechanical and metabolic conditions, caused no significant change in contractility. The mean change in force was 1% ± 7% in nine different trabeculae from nine different hearts. It is unlikely that the loss of the effect of the effluent on the contractility of the trabecula is due to removal of a diffusion barrier as the effect is in the wrong direction. It appears that the presence of the endothelial cells in the trabeculae is necessary for the signals in the coronary venous effluent to have an effect on contractility of the trabeculae.

**DISCUSSION**

The ability of the coronary effluent to lower as well as raise peak force in the trabecula suggests that a combination of factors with opposing effects is likely to be released by the endothelial cells in the trabeculae. This situation has already been demonstrated for vasoactive substances produced by vascular endothelium by the use of specific inhibitors of the activity of nitric oxide to unveil the presence of an agent producing contraction of smooth muscle at the same time (15).

From these studies of the response of cardiac cells to the coronary venous effluent of a perfused heart, it appears that cardiac myocytes or some other cellular component of the tissue releases substances by a mechanism that is sensitive to the metabolic and the mechanical states of the myocyte. The substances interact with endothelial cells, presumably those in the arterioles and possibly venules but probably not capillaries, to stimulate the release of factors that regulate cardiac contractility. Indirect evidence in favor of this phenomenon has already been generated (12, 16). Such a mechanism would provide a feedback loop that could reduce the contractile activity of the myocardial cells when the energy supply is reduced and increase contractile activity when the load on the heart is increased and the oxygen supply is adequate. The sensors could operate at the cardiac myocyte and the information processing could occur at the endothelial cells. Coronary vascular tone has already been shown to be influenced by the rate of oxidative phosphorylation in the myocytes (17). Catecholamines are very unlikely to be directly involved in producing the change in contraction inasmuch as they cause very different changes in the shape of the contraction from those observed in those studies; they increase the rate of rise of force, shorten the contraction, and accelerate the rate of relaxation.

The nature of the signal substances added to the coronary perfusion fluid is not clear. The substances are unlikely to be highly labile in view of the time course of the experiments. It is clear, however, that the endothelial cells are required for the signal substances in the coronary venous effluent to have an effect, and in view of the short diffusion distances there should be no limitations imposed by lability of the factors released within the trabeculae by the endothelial cell.

The identity of the regulatory endothelial factors remains obscure. The factors may be among those already identified as active in the regulation of smooth muscle by endothelial cells. There is a certain amount of indirect data supporting this notion, and endothelin has already been shown to have a powerful effect on the contraction of cardiac myocytes (6, 7). On the other hand, there is other evidence that endothelial cells may produce as yet unidentified factors that alter cardiac contractility (11).

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