Metabolic communication from cardiac myocytes to vascular endothelial cells

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Metabolic communication from cardiac myocytes to vascular endothelial cells. Am J Physiol Heart Circ Physiol 288: H2232–H2237, 2005; doi:10.1152/ajpheart.00202.2004.—The endothelium releases substances that affect both vascular and cardiac myocytes. However, under conditions of augmented metabolic demands and cardiac work, signals from the cardiac myocytes may be critical for the endothelium to fulfill its secretory and regulatory function in the vascular bed. Therefore, we hypothesized that cardiac myocytes produce substances that alter the resting membrane potential of endothelial cells and thus vascular tone. Isolated rat cardiac myocytes were electrically stimulated at the rate of 0 and 400 beats/min (P02 = 150 mmHg), and supernatants were collected from each group (Sup-0; control) and (Sup-400) and used within 6 mo. These supernatants were applied to human coronary endothelial cells that were subsequently analyzed by using the whole cell and cell-attached patch-clamp modes. Sup-0 had no effect on the whole cell current and the zero-current potential. The Sup-0 from myocytes treated with aprotinin, an inhibitor of kallikrein and serine protease, reduced whole cell current and increased the magnitude of an inward current, and activated an outward current. Moreover, Sup-400 cells assayed in cell-attached patches increased single channel amplitude and the probability of a channel being in the open state. These effects were reversed by the Sup-400 from aprotinin-treated cells. We conclude that under certain metabolic conditions, isolated cardiac myocytes produce and release vasoactive substances that alter the function of K+ channels in vascular endothelial cells. Thus cardiac myocytes seem to communicate metabolic information to the endothelium, which could potentially influence vascular tone.

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hearts were perfused by using modified Langendorff apparatus. The calcium-tolerant cardiac myocytes were isolated by perfusion with collagenase. Only preparations containing at least 70% of rod-shaped cells were used for the further studies (for details, see Ref. 9).

Myocytes (125,000 per 1 ml) were stimulated for 20 min at the rate of 0 (Sup-0), 200 (Sup-200), and 400 beats/min (Sup-400) in a custom-designed chamber (2 ml) at room temperature. Myocytes were stimulated in the physiological buffer of a composition similar to bath solution used in patch-clamp experiments and osmolality of 300 mosmol/l. The osmolarity of this buffer was not altered after the stimulation of myocytes. Myocytes were separated by gentle centrifugation (2 min, 5,000 rpm). The supernatant was aliquoted (100 μl), snap frozen on dry ice, and stored at −80°C (maximum up to 6 mo) for assay of vasoactive substances. Oxygen consumption was measured by stimulating myocytes in an airlight chamber (2 ml), withdrawing supernatant at fixed time points, and analyzing it in an ABL-5 automated gas analyzer (Radiometer).

Cell culture. Human coronary artery endothelial cells were purchased from Clonetics (San Diego, CA) and were subcultured in EGM-2MV medium (Clonetics) with 2% FBS. The cell culture was maintained at 37°C in humidified atmosphere of 5% CO2 in air. For patch-clamp experiments, cells were detached by scraping with the sterile cell scraper.

Electrophysiology. Measurements of ionic currents were performed by using patch-clamp technique in whole cell and cell-attached configuration (6). The whole cell measurements were obtained by using high-K+ solution in the pipette and PSS in the bath. In cell-attached experiments, the bath and the pipette solution was PSS. Recording pipettes were pulled from borosilicate glass (cat. no. 7052; Garner Glass, Claremont, CA) using a vertical puller (Narashige, Tokyo, Japan) and had resistance of 2–4 MΩ when filled with high-K+ solution. Voltage protocols and data acquisitions were carried out by using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) and a Digidata 1200A analog-to-digital converter (Axon Instruments). Currents were filtered by a four-pole Bessel filter at 1 kHz and digitized at 5–10 kHz. The indifferent electrode was an Ag-AgCl plug electrically connected to the bath via 140-mM KCl agar bridge. During acquisition, capacitative transients were not compensated. The patch-clamp experiments were performed at room temperature.

Measurements were made in endothelial cells under basal control conditions, after administration of 100 μl of supernatant from the quiescent or stimulated myocytes. At the day of experiment, supernatant stored at −80°C was transferred to −20°C and thawed just before the addition to the 1 ml patch-clamp bath. Supernatant was administered to human coronary artery endothelial cells from two different human donors. The donors did not suffer from heart or coronary artery disease according to the manufacturer’s information.

Aprotinin, a nonspecific serine protease inhibitor, has been used in vitro studies to inhibit various proteases including those from kinin-kallikrein system. The use of such an inhibitor allowed evaluation of whether a product in the supernatant was derived from proteolysis in the myocyte preparation under the various conditions or was a product of the cardiac oxidative metabolism. In the first set of experiments, aprotinin [20 U/ml, 20 min (8)] was administered to the myocytes, and this supernatant was then applied to the endothelial cells. This caused a dilution of the aprotinin that was estimated to be ~5 U/ml. To determine a direct effect of aprotinin on the plasma membrane of vascular endothelial cells, aprotinin (10 and 1 U/ml) was added to the bath solution. The recordings were taken 5, 10, and 20 min after the addition. The data are presented after 20 min of exposure to aprotinin. The voltage protocol applied in this set of experiments was altered in 5-mV increments.

PSS contained (in mM) 145 NaCl, 5.4 KCl, 1.8 CaCl2, 1 MgCl2, 5 HEPES, and 10 glucose (pH = 7.4 adjusted with NaOH). The standard pipette solution contained (in mM) 145.0 K+-aspartate, 5.0 NaCl, 0.3 CaCl2, 2.2 EGTA, 10 HEPES, and 7.5 glucose (pH = 7.2 adjusted with KOH). Osmolarity of solutions was maintained between 280 and 300 mosmol/l.

Data analysis. Analysis of whole cell data was carried out by using pCLAMP 6.0 software (Axon Instruments). Unitary reversal potentials and conductance values were estimated by fitting the linear portion of each current-voltage (I-V) relationship. Data represent direct comparison between control and treated cells. Differences within and between groups were determined by using ANOVA for repeated measurements. A value of P < 0.05 was considered statistically significant.

RESULTS

Quiescent myocytes consumed 23 ± 15 nl O2·min⁻¹·100,000 cells⁻¹, and this value increased 41-fold with an increase in the stimulation up to 400 beats/min (9). Supernatant (100 μl) from quiescent cardiac myocytes and those paced at 400 beats/min was added to the human coronary artery endothelial cells. Whole cell current was recorded in response to voltage steps, using PSS in the bath and high-K+ solution in the pipette. Under this condition, control cells displayed a zero-current potential reversal potential (|Erev|) of −45 ± 2 mV (n = 5), a value consistent with that previously reported for macrovascular human coronary artery endothelial cells (19). Control records (Fig. 1A) and the corresponding I-V relationship (Fig. 1C) showed that current rectified inwardly at the potentials hyperpolarized from −60 mV. This result suggests that whole cell current in the control was carried through inward rectifier K⁺ (KIR) channels known to be the predominant K⁺ channels in vascular endothelial cells.

Current recorded from endothelial cells treated with supernatant from the quiescent myocytes (Sup-0, 100 μl) was not statistically different from the control currents (Fig. 1B), with |Erev| remaining otherwise unaltered (n = 5, Fig. 1C).

Currents recorded from endothelial cells treated with supernatant from myocytes stimulated at 200 beats/min (Sup-200, 100 μl, n = 7) were only slightly increased, with |Erev| shifted to the more positive value (Fig. 1D). However, the outward current was statistically increased (n = 7, P < 0.05).

In contrast to the results from unpaced myocytes, currents recorded from cells treated with Sup-400 (100 μl; 15 min) were larger in magnitude in both inward (n = 7, P < 0.05, Fig. 2C) and outward directions (n = 7, P < 0.05, Fig. 2C). The I-V relationship corresponding to the currents was almost linear between the potential of −40 and +40 mV (Fig. 2C), with |Erev| equal to −5 ± 1 mV (n = 7), a value significantly depolarized from control (P < 0.05). The increased overall current suggests that the Sup-400 activates a whole cell current in vascular endothelial cells.

To determine the possible contribution of the vasodilator compounds to the changes in the whole cell current induced by Sup-400, the nonspecific inhibitor of kallikrein, serine protease, and aprotinin were administered to myocytes when they were quiescent or stimulated at 400 beats/min. Currents recorded from cells treated with Sup-0 + aprotinin were smaller in magnitude in the inward direction (n = 3, P < 0.001, Fig. 3C), but the corresponding I-V relationship displayed the |Erev| value unchanged compared with the control. The inward rectification observed under control conditions was maintained after the addition of Sup-0 + aprotinin.

In contrast to the control responses observed after administration of Sup-0 + aprotinin, currents recorded from cells treated with Sup-400 + aprotinin showed a dramatic difference...
in the activation of the whole cell current. The Sup-400 + aprotinin depolarized the cells and shifted $E_{rev}$ to the value of $-5$ mV, a value only slightly depolarized (10 mV) from the control ($-45$ mV). The $I-V$ relationship corresponding to these currents was essentially linear (Fig. 4C); the magnitude of the current was only slightly different from the control values in the inward direction but was dramatically altered in the outward direction ($n = 3$, $P < 0.001$). However,
the inward rectification observed under control conditions was completely abolished, suggesting the possibility that Sup-400 + aprotinin-activated current is either different from the control KIR or Sup-400 + aprotinin has a profound effect on KIR channel function. Aprotinin alone had no effect on a magnitude of the whole cell current (n = 5, Fig. 5).

Cell-attached membrane patches were made on the control cells; the control channel activity was measured before cells were treated with Sup-400 or Sup-400 + aprotinin, respectively (Fig. 6). The pipette solution and bath solution were PSS. Channel activity was resolved only at −70 mV, and −120 mV, respectively. Under control conditions, at −120 mV, channel amplitude was −1.31 ± 0.28 pA (n = 5). The addition of Sup-400 increased channel amplitude to −6.55 ± 0.43 pA (n = 10, P < 0.001), whereas the addition of the Sup-400 + aprotinin did not affect channel amplitude (amplitude −1.31 ± 0.1 pA, n = 10), respectively (Fig. 6A). The channel activity followed a similar pattern at −70 mV. Under control conditions, the recorded amplitude was −0.69 ± 0.08 pA (n = 5) and was increased to −3.55 ± 0.40 pA (n = 10, P < 0.003) on the addition of the Sup-400. Addition of the Sup-400 + aprotinin had no effects on the amplitude of ion channel (Fig. 6B).

DISCUSSION

The major new finding in this study is that cardiac myocytes produce substances that impact directly on electrophysiological parameters in endothelial cells. Thus there is a pathway of communication from cardiac myocytes to the vascular endothelium. Specifically, we observed that cardiac myocytes produce a factor or factors that affect whole cell current and modulates action of the KIR ion channels, and this pathway is affected by metabolism. In this way, metabolic signals have the potential to be transmitted from cardiac myocytes to endothelial cells. This indicates a possibility that under various phys-
endothelial cells from papillary muscles affects the contractility of the muscle, indicating that the endothelium needs to be present for the muscle to function properly (9, 13, 14). Additionally, the communication between endothelial cells and the cardiac myocytes regulates endothelial cell gene expression in vivo, ex vivo, and in vitro. Specifically, myocytes induced expression of von Willebrand factor in cardiac microvascular endothelial cells. Moreover, exposure of cardiac myocytes to hypoxia induces upregulation of PDGF receptors in endothelial cells. Thus cardiac myocytes have the ability to alter the endothelial cell phenotype.

The physical proximity of endothelial cells and cardiac myocytes may suggest a possibility of bidirectional transfer of metabolic information according to the environmental conditions and the intensity of the cardiac work. The supernatant derived from quiescent cells generated no effects on the whole cell current in endothelial cells. This indicated that under such circumstances of low cardiac metabolism, the production of cardiac vasoactive factors may be limited or insufficient to exert profound changes, and we speculate that with low cardiac metabolism, endothelial cells could control the function of the arterioles. It has to be recognized that the situation under which cardiac myocytes remained quiescent or contract only spontaneously is not physiological, yet after administration of aprotinin to cardiac myocytes, quiescent myocytes produced a factor that reduced whole cell current. This finding provides important information indicating cardiac myocytes are able to secrete vasoactive compounds even under minimum workload when the conditions are favorable. The addition of aprotinin appears to alter the metabolic balance and homeostasis between the cardiac myocytes and the endothelial cells.

Electrical pacing at 400 beats/min of cardiac myocytes is associated with an enormous increase in the oxygen consumption, which may activate metabolic pathways to produce metabolic dilators to dilate coronary resistance vessels (9) and thus augmented oxygen delivery and flow. However, we found that this condition was also associated with factors that depolarize endothelial cells. This level of depolarization to −5 mV, produced by closure of the KIR channels, would have a tremendous effect on endothelial cell metabolism and production of vasoactive factors. In endothelial cells, the KIR channel (2.1–2.3) family plays an extremely important role in the regulation of the resting membrane potential and hence the

Fig. 5. Aprotinin alone had no effects on whole cell current magnitude. Control currents were recorded in response to successive voltage pulses from −100 to +100 mV, in 5-mV increments. No statistical significance was determined between whole cell currents recorded under control conditions and after treatment with aprotinin. N = 5 cells.

Fig. 6. Single channel currents in vascular endothelial cells under control conditions and after Sup-400 + aprotinin treatment. A: single channel amplitude was determined at −120 mV under control conditions (n = 5) after the addition of 400 beats/min (n = 10, P < 0.001 vs. control) and 400 beats/min + aprotinin (n = 5, not significant vs. control). B: single channel amplitude was recorded at −70 mV under control conditions after the addition of 400 beats/min (n = 10, P < 0.003 vs. control) and 400 beats/min + aprotinin (n = 5). Data points represent mean values at each potential; error bars represent ± SE.
function of the blood vessels (12, 18). The resting membrane potential in endothelial cells maintains a gradient for the Ca\(^{2+}\) influx and controls the production of nitric oxide. Because Sup-400 significantly depolarized the endothelial cell’s resting membrane potential, there would be a large reduction of Ca\(^{2+}\) influx. The linear characteristic of the Sup-400-activated current indicates a possibility of activation of a current different from K\(_{IR}\) or an alteration in the K\(_{IR}\) that leads to a loss of inward rectification. This is an unresolved question because the Sup-400 + aprotinin repolarized the cell, but magnitude of the outward current remained unaffected. The loss of inward rectification may imply an impairment of the function of the K\(_{IR}\) ion channel and the loss of its ability to control the resting membrane potential of endothelial cells.

We would like to mention another caveat about our experimental preparations. Our interpretation of results during electrical stimulation is on the basis of the increase in oxygen consumption, but we cannot eliminate the possibility that the cells may have become hypoxic during the increase in oxygen consumption. This possibility is based on the fact that the cells were consuming large amounts of oxygen, but the oxygen content of the buffer is negligible; thus there may be local hypoxia in the region of the myocytes in the bath. We will admit that we do not believe hypoxia occurred, because oxygen would then be rate limiting and oxygen consumption would not have increased so robustly during electrical stimulation.

Depolarization of endothelial cells, and thus decreases in the production of nitric oxide, occur at a time when metabolic needs of the myocardium are greatest, during increases in cardiac work. Although this seems counterintuitive, such an effect may be beneficial from the aspect of which cell type controls coronary blood flow and under which condition. For example, when cardiac metabolism is low, and the cardiac production of vasoactive factors is low, then the dominant control may be exerted by the endothelium. However, when myocardial metabolism is augmented, unknown cardiac factors depolarize endothelial cells, which essentially remove endothelial control from the equation, and thus coronary blood flow is predominantly controlled by cardiac metabolism. This view is supported by several observations showing that inhibition of nitric oxide synthase affects coronary blood flow at baseline, but typically does not affect flow during augmented cardiac metabolism.

Taken together, an interaction of the two different cell types at the ion channel level seems possible and relevant under physiological and pathophysiological conditions. The open question remains, where and how the cells switch so that during variations in cardiac work a signal from one cell type may be able to overcome that of the other.

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