Pattern differences between distributions of microregional myocardial flows in crystalloid- and blood-perfused rat hearts

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Matsumoto, Takeshi, Hiroyuki Tachibana, Takahisa Asano, Mami Takemoto, Yasuo Ogasawara, Keiji Umetani, and Fumihiko Kajiya. Pattern differences between distributions of microregional myocardial flows in crystalloid- and blood-perfused rat hearts. Am J Physiol Heart Circ Physiol 286: H1331–H1338, 2004. First published December 11, 2003; 10.1152/ajpheart.00120.2003.—Regional myocardial flow distributions in Langendorff rat hearts under Tyrode and blood perfusion were assessed by tracer digital radiography (100-μm resolution). Flow distributions during baseline and maximal hyperemia following a 60-s flow cessation were evaluated by the coefficient of variation of regional flows (CV; related to global flow heterogeneity) and the correlation between adjacent regional flows (CA; inversely related to local flow randomness). These values were obtained for the original images (642 pixels) and for coarse-grained images (322, 162, and 82 blocks of nearby pixels). At a given point in time during baseline, both CV and CA were higher in blood (n = 7) than in Tyrode perfusion (n = 7) over all pixel aggregates (P < 0.05, two-way ANOVA). During the maximal hyperemia, CV and CA were still significantly higher in blood (n = 7) than in Tyrode perfusion (n = 7); however, these values decreased substantially in blood perfusion and the CV and CA differences became smaller than those at baseline accordingly. During basal blood perfusion, the 60-s average flow distribution (n = 7) showed a smaller CV and CA than those at a given point in time (P < 0.05, two-way ANOVA). Coronary flow reserve was significantly higher in blood than in Tyrode perfusion. In conclusion, the flow heterogeneity and the local flow similarity are both higher in blood than in Tyrode perfusion, probably due to the different degree of coronary tone preservation and the presence or absence of blood corpuscles. Under blood perfusion, temporal flow fluctuations over 60-s order are largely involved in shaping microregional flow distributions.

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THE ISOLATED HEART MODEL USING crystalloid perfusates has been extensively used for characterizing myocardial contractility and energetics and the distribution of regional myocardial flows as well (5, 18, 49). However, most of the knowledge on regional myocardial flows could not be extrapolated to explain the mechanisms of local regulation and coordination of regional myocardial flows under blood perfusion. Low O2 carrying capacity of crystalloid perfusates makes an intracellular O2 tension inadequate or just above the threshold for impaired myocardial function (6, 38) and reduces coronary tone or reserve (12, 28, 34). The distribution of regional myocardial flow is greatly under the influence of coronary tone (2, 5), and therefore there will be differences between spatial flow patterns in crystalloid- and blood-perfused myocardium. Furthermore, the presence or absence of blood corpuscles will augment those differences, because rheological, stochastic, and functional behaviors of corpuscles alter regional flows greatly at microvascular beds (8, 9, 15, 17, 43).

Thus the present study was undertaken to clarify the differences between spatial patterns of regional myocardial flows in crystalloid- and blood-perfused isolated rat hearts. The within-layer flow distribution of the left ventricle (LV) was measured during baseline and maximal hyperemia by quantitative digital radiography combined with a tritiated desmethylimipramine (HDMI) deposition technique (29, 30). This technique makes it possible to evaluate the flow distribution with resolving the tissues area into 100 × 100-μm2 regions, comparable in size to the region supplied by a single arteriole (3). The patterns of flow distribution during baseline and maximal hyperemia were quantitated by the coefficient of variation of regional flows (CV) and the correlation between adjacent regional flows (CA).

MATERIALS AND METHODS

Experiments using male Wistar rats were conducted in accordance with the Guiding Principles of the American Physiological Society (1a) and with prior approval of the Committee on Animal Research of Kawasaki Medical School.

Tyrode perfusion. A rat (10–14 wk, n = 14) was anesthetized with diethyl ether inhalation and anticoagulated with intravenous heparin injection (10,000 U/kg). The heart was isolated according to the Langendorff technique and perfused with filtered Tyrode’s solution with a head pressure of 100 mmHg. Perfusion was maintained at 37°C in a water-jacketed reservoir and gassed at 95% O2-5% CO2; pH was adjusted to 7.35–7.40 with 1 M NaOH. The preparation was completed within 30 s while keeping the heart beating. Bovine serum albumin (10%) was infused into the perfusion line at a 100th coronary perfusion rate to achieve a perfusate concentration of 0.1% (18). The LV cavity was cannulated via its apex and communicated with the atmosphere.

Blood perfusion. A large support rat (>500 g, 14–18 wk) was anesthetized with intraperitoneal administration of pentobarbital sodium (30 mg/kg) and injected intravenously with heparin (10,000
U/kg. The rat was placed supine on a 37°C heating plate. Ventilation was maintained via a tracheotomy and with a mixture of \( \text{O}_2 \) and room air, adjusted to keep arterial pH and arterial blood gases within physiological limits (pH 7.35–7.45; \( \text{PCO}_2 \) 35–45 mmHg; \( \text{PO}_2 \) 100–200 mmHg). Sodium bicarbonate was infused intravenously as the occasion demanded. A carotid artery and a femoral vein were isolated and cannulated with Teflon catheters. The arterial catheter was connected to a 37°C water-jacketed reservoir into which arterial blood was pumped with the LV ejection pressure. The overflow was conveyed to another 37°C water-jacketed reservoir connected to the venous catheter (Fig. 1). The tube line and the reservoirs were filled in advance with blood obtained from a blood donor rat to avoid hypovolemia of the support rat.

In parallel with the support rat preparation, the heart was isolated from an anesthetized rat (10–14 wk, \( n = 21 \)) in the same manner as described in Tyrode perfusion and perfused with oxygenated blood from the arterial-side reservoir. Head pressure was maintained at 100 mmHg by continuous overflow to the venous-side reservoir. The coronary effluent was collected and transferred to the venous-side reservoir manually with a syringe. Arterial pH and arterial blood gases were checked at frequent intervals.

Instrumentation. A small handmade latex balloon connected to a manometer catheter (Camino Labs) via a polyethylene (PE)-50 tube was used for isovolumic LV function measurements. The balloon was inserted into the LV through the mitral valve via an incision in the left atrium. The LV pressure signal was amplified with a pressure transducer (Camino Labs). The balloon was inflated with saline to adjust the end-diastolic pressure at 5–10 mmHg. Under the assumption of the first-order time lag, the time constant of the pressure measurement was estimated as 16.5 ms. Perfusion rate was measured in the aortic flow line with a transit-time ultrasonic flowprobe and a flowmeter (Transonic Systems). The heart was put in the 37°C mist of warmed saline generated by a nebulizer and paced at 300 beats/min by a pacemaker (Medtronic) through platinum wires sutured to the right atrium and the right ventricular apex. All measured signals were recorded with a sampling rate of 200 Hz using a PC (Macintosh G3) with LabVIEW (National Instruments). The heart was allowed to stabilize before data acquisition.

Flow tracer injection and sample preparation. In Tyrode-perfused hearts (\( n = 7 \)), 2 \( \mu \text{Ci} \) of HDMI (New England Nuclear Life Science) was injected into the perfusion line immediately above the aortic root over a short period of 1–2 s with a microsyringe. In blood-perfused hearts, the same amount of HDMI was injected as a bolus (blood-bolus-perfused hearts; \( n = 7 \)) as in Tyrode-perfused hearts and injected over a period of 60 s (blood-60 s-perfused hearts; \( n = 7 \)). The tracer injection did not disturb coronary hemodynamics. Before the tracer injection, reactive hyperemia after a 30-s flow cessation was elicited to evaluate coronary flow reserve. A percentage of maximal flow increase after reperfusion to basal flow was recorded.

Additionally, the same amount of HDMI was injected as a bolus during the peak plateau of the reactive hyperemia after a 60-s flow cessation under Tyrode perfusion (Tyrode-reperfused hearts; \( n = 7 \)) and under blood perfusion (blood-reperfused hearts; \( n = 7 \)). Coronary flow reserve was recorded as denoted above. These experiments were done with 300 beats/min pacing but without the intraventricular balloon insertion.

The heart was allowed to beat for 20 s after the completion of the tracer injection. The heart was then arrested with a saturated KCl solution and weighed. Our preliminary experiment showed that the myocardial HDMI retentions at 20 and 80 s after the tracer injection under Tyrode and blood perfusion, respectively, were both nearly 95%. Thus the HDMI deposition in a certain small piece of tissue can be assumed to be proportional to the accumulated flow perfused into that piece. The bolus injection of HDMI yields the flow distribution at a given point in time, whereas the 60-s continuous injection of HDMI yields the distribution averaged for 60 s. The HDMI density distributions obtained from Tyrode- and blood-reperfusion hearts would approximate the relative flow distributions under maximal vasodilation, because the 60-s flow cessation was confirmed to be sufficient to induce maximal hyperemic flow under either perfusion.

The full-wall-thickness sample was excised from the LV equatorial portion. After the removal of papillary muscles, the sample was sandwiched in aluminum sheets and immediately put into a \(-80°C \) freezer. The frozen sample was then divided into 10-\( \mu \)m-thick slices from subendocardium to subepicardium using Tissue-Tek tissue mount in a cold environment (\(-25°C \)) of cryostat microtome (Zeiss). Through this process, layers including some large coronary vessels or warping due to the influence of uneven endocardial surface were omitted. Slices were carefully put onto a slide glass and checked to ensure that no wrinkles had been made. Finally, 28 slices were picked equally throughout the layer and intended for digital radiographical measurements.

Digital radiography. The digital radiographical technique for within-layer flow visualization was described in detail in our previous publications (29, 30). In brief, each slice was exposed to the tritium-sensitive radioactive energy sensor (Fujix) for 3 days. The radioactive energy distribution, i.e., the relative flow distribution, was converted into 10-bit digital data of 100-\( \mu \)m pixels by the imaging analyzer (Fujix) and visualized with a 1,024-step gradation. The mean background density was <5% of that of a region overlying the tissue. Corrected for background activity, a 64 \times 64-pixel portion of each image was ready for the data analysis through our image processing system using a PC (Macintosh G3) with MATLAB (MathWorks).

Characterization of a spatial flow pattern. Two indices, CV and CA, were used for characterizing regional flow distributions. CV was defined as (SD of regional flows)/mean flow, related to global flow heterogeneity on the relative basis. CA is the correlation between adjacent regional flows or the local flow similarity, which is given by

\[
CA = \frac{|V \sum C(d) + C(\sqrt{2d})(1 + \sqrt{2})|}{C(d)}
\]

with \( C(d) = \langle |m_i - m_j| \rangle - \langle m_i \rangle \langle m_j \rangle \rangle / \left( \langle m_i^2 \rangle - \langle m_i \rangle \langle m_i \rangle \right) \rangle \rangle \) where \( m \) is the HDMI density of the \( i \) pixel of a digital radiogram and \( < \) denotes an ensemble average over all the pairs of \( i \) and \( j \) pixels, the centroids of which are in a certain region. Here, \( d \) is set to the pixel size, which is 100 \( \mu \)m for the present digital radiogram. When \( CA \) is close to one, flows are distributed with shaping high- or low-flow domains locally; when it is close to zero, nearby flows are rather randomly distributed.
These two indices depend on the resolved region size. To examine the resolution-dependence of the perfusion pattern, CV and CA were also computed for coarse-grained perfusion images described with \(2 \times 2\), \(4 \times 4\), or \(8 \times 8\)-square blocks of nearby pixels (pixel aggregates). In the calculation of CA, \(d\) was set at 200, 400, and 800 \(\mu m\) according to the size of a pixel aggregate.

Statistics. Between-group differences in hemodynamic variables were assessed with the unpaired \(t\)-test. Two-way ANOVA for repeated measures was used to analyze between-group differences in CV and CA related to the size of a pixel aggregate. A value of \(P < 0.05\) was considered statistically significant in all testing. Data are represented as means \(\pm SD\).

RESULTS

In Tyrode-perfused hearts, coronary perfusion rates and percent flow increases after 30-s flow cessation were 13.6 \(\pm 2.7\) ml min\(^{-1}\)g\(^{-1}\) and 15 \(\pm 9\%\), respectively; in blood-perfused hearts, these were 2.6 \(\pm 0.3\) ml min\(^{-1}\)g\(^{-1}\) and 77 \(\pm 14\%\), respectively. There were substantial differences in these variables between the two groups (\(P < 0.05\), unpaired \(t\)-test). These variables, however, did not differ between blood-bolus- and blood-60 s-perfused hearts. No difference was found in LV peak-systolic and end-diastolic pressures between Tyrode- and blood-perfused hearts (113 \(\pm 17\) vs. 113 \(\pm 19\) mmHg and 7 \(\pm 1\) vs. 6 \(\pm 1\) mmHg).

In Tyrode-perfused hearts, coronary perfusion rates and percent flow increases after 60-s flow cessation were 15.6 \(\pm 2.5\) ml min\(^{-1}\)g\(^{-1}\) and 17 \(\pm 5\%\), respectively. These were significantly different from those of blood-perfused hearts, which were 3.1 \(\pm 0.4\) ml min\(^{-1}\)g\(^{-1}\) and 133 \(\pm 34\%\), respectively (\(P < 0.05\), unpaired \(t\)-test). The higher basal perfusion rates in Tyrode- and blood-perfused hearts than in Tyrode- and blood-perfused hearts were attributed to the lack of LV balloon insertion. On the other hand, the longer flow cessation period (60 vs. 30 s) resulted in the higher percent flow increases in blood-perfused than in blood-perfused hearts but not in Tyrode-perfused hearts, indicating that nearly maximal coronary vasodilation was already induced under Tyrode perfusion after reperfusion after a 30-s flow cessation.

Figure 2 (top) shows a typical example of within-layer distributions of normalized HDMI density (mean = 1) in Tyrode- and blood-perfused hearts; that is, these represent flow distributions on the relative basis. Shading in each \(100 \times 100\)-\(\mu m\) region is proportional to flow: a darker region received higher flow. The flow heterogeneity is the lowest under Tyrode perfusion and the highest under blood-bolus perfusion. In Fig. 2 (bottom), regional flows in each distribution above are classified in three categories: high, middle, and low. High-flow regions of two or more times higher than the mean flow are black, low-flow regions of less than half the mean are white, and the others are gray. In the distribution under blood-bolus perfusion, the low-flow domains are clearly perceived from the lower left to the upper right and the high-flow domains on the left; however, such a distinct low- or high-flow domain did not appear in the distribution under Tyrode perfusion. The flow distribution under blood-60 s perfusion is intermediate between the two extremes.

In Fig. 3, an index of flow heterogeneity (CV) is plotted against the size of a pixel aggregate for Tyrode-, blood-bolus-, and blood-60 s-perfused hearts. There was an overall difference in CV between the three groups (\(P < 0.05\), ANOVA); CV was the lowest in Tyrode-perfused hearts and the highest in blood-bolus-perfused hearts. By increasing the size of a pixel aggregate from 100 to 800 \(\mu m\), CV decreased from 0.45 \(\pm\) 0.12 to 0.19 \(\pm\) 0.06 in Tyrode-perfused hearts, from 0.73 \(\pm\) 0.11 to 0.50 \(\pm\) 0.12 in blood-bolus-perfused hearts, and from 0.67 \(\pm\) 0.08 to 0.35 \(\pm\) 0.07 in blood-60 s-perfused hearts. The decrement of CV by increasing the size of a pixel aggregate was smaller at a larger pixel aggregate.

It is apparent from Fig. 2 that the distribution of regional flows is far from random. Actually, an index of the correlation between adjacent regional flows, CA, was significantly higher than zero as shown in Fig. 4. There was an overall difference in CA among the three groups (\(P < 0.05\), ANOVA); CA was the lowest in Tyrode-perfused hearts and the highest in blood-bolus-perfused hearts. As was the CV difference, CA of blood-60 s-perfused hearts was intermediate between the two
extremes. These indicate that regional flows are most randomly distributed in Tyrode-perfused hearts and that there exist domains consisting of similar low- or high-flow regions to the greatest extent in blood-bolus-perfused hearts. There was a similarity in the dependence of CA on the size of a pixel aggregate among three groups; CA seemed to reach the peak when the size of a pixel aggregate was 200 or 400 μm or between them.

Figures 5 and 6 show CV and CA, respectively, plotted against the size of a pixel aggregate for Tyrode- and blood-reperfused hearts. The overall differences in CV and CA were significant (P < 0.05, ANOVA); both CV and CA were lower in Tyrode- than in blood-reperfused hearts. However, the differences in CV and CA were smaller than those between Tyrode- and blood-bolus-perfused hearts. That is, according to the change from basal to maximal vasodilated states, CV and CA decreased largely under blood perfusion, but varied only slightly under Tyrode perfusion.

DISCUSSION

In the present study, we clarified the difference in the distribution of microregional myocardial flows between crystalloid (Tyrode)- and blood-perfused rat hearts. The present findings are as follows. First, the within-layer flow distribution under Tyrode perfusion was characterized by low CV and CA compared with those of the distribution under blood perfusion, which translates into flows with lower heterogeneity and locally at random to a higher degree under Tyrode perfusion. Second, under blood perfusion, CV and CA were both smaller in the flow distribution averaged for 60 s than those in the distribution at a given point in time. Third, during maximal hyperemia, both CV and CA decreased largely under blood perfusion but varied only slightly under Tyrode perfusion, resulting in decreases of CV- and CA-differences between flow distributions under Tyrode and blood perfusion although the differences were still significant.

Tyrode vs. blood perfusion. Basal perfusion rate was five times higher in Tyrode-perfused than in blood-perfused hearts, whereas the O₂ content in oxygenated Tyrode solution is only 0.02 ml O₂/ml buffer or the one-tenth O₂ content in blood even at high P0₂ of >600 mmHg (1). Consequently, the O₂-carrying capacity of Tyrode solution was half that of arterial blood and accordingly, myocardial oxygenation would be marginal as indicated in the measurement of myoglobin O₂ saturation under Krebs perfusion (38). Thus coronary tone will be less preserved in Tyrode than in blood perfusion. Furthermore, the increased perfusion rate due to the limited oxygenation and the lack of corpuscular flow resistance under Tyrode perfusion will also attenuate the coronary tone through the increased...
release of vasodilatory factors via augmented shear stress on the endothelium (33, 36). The decreased coronary tone under Tyrode perfusion was also confirmed by the lower flow increase rate during hyperemia in Tyrode than in blood perfusion. For reference purposes, we showed coronary microangiograms of Langendorff rat hearts under Krebs perfusion (pacing 300-beats/min; head pressure 100 mmHg) with and without canine erythrocytes (Fig. 7). The erythrocyte-containing solution (40% Hct) gassed at 20% O₂, 75% N₂, and 5% CO₂ imitated arterial blood. Coronary arteriolar diameters were larger under erythrocyte-free Krebs perfusion, implying less preserved coronary tone when perfused with crystalloid solutions than with blood. The difference of coronary tone (2, 5), and additionally, dynamical flow behaviors of corpuscles (8, 9) and vasodilatory signals released from erythrocytes (15, 43) would all participate in causing different flow distributions under crystalloid and blood perfusion.

Tyrode- and blood-perfused hearts showed similar LV pressure waveforms (data not shown) with similar diastolic and systolic pressures. This similarity could be interpreted that cardiac mechanical effects on regional myocardial perfusion were also similar under Tyrode and blood perfusion. A compliant property of LV pressure transmission path to a manometer catheter will reduce the time responsibility of pressure measurement (the time constant of 16.5 ms) and accordingly, diastolic and systolic LV pressures would be over- and underestimated, respectively. However, the simultaneous LV pressure measurements with connecting a manometer to the balloon via PE-50 tube (present method) and with placing the tip of a manometer within the balloon showed that the present system underestimates the peak-systolic pressure only by 6% and estimates the end-diastolic pressure fairly accurately even under 300-beats/min pacing. Accordingly, the similar LV pressure waveforms imply that the cardiac mechanical effects on flow distributions would also be similar, and therefore the involvement of cardiac mechanical stress in flow pattern differences could be neglected in the present study.

Recent studies (22, 35) showed that endothelial glycocalyx plays a significant role in vascular endothelial function. Accordingly, the modulation of its integrity under Tyrode perfusion might lead to endothelial dysfunction and could be partly attributed to the CV and CA differences through coronary tone alterations. However, in the present study, Tyrode solution contained 0.1% bovine serum albumin, the concentration of which seems suitable for preservation of a hairy-like structure of glycocalyx (44). Thus the glycocalyx-dependent endothelial function seemed intact in the present study.

Spatial pattern of regional flows. Regional myocardial flow heterogeneity, i.e., CV, is likely to be related inversely to perfusion rate when myocardial O₂ availability is limited (10, 30, 40, 47). In Tyrode-perfused hearts, perfusion rate is high, but myocardial oxygenation is limited and consistent with those earlier studies, CV was lower than that in blood-perfused hearts (Fig. 3). The mechanism of CV decrease accompanying high perfusion rate with the limited O₂ availability remains unclear; however, it is reasonably assumed that the decreased CV is attributed to a reduced variability of regional flow resistance or arteriolar diameter, resulting from the coronary tone attenuation. Figures 3 and 5 indicate that the abolishment of coronary tone decreased CV largely under blood perfusion but not under Tyrode perfusion, suggesting the substantial role of coronary tone in determining the degree of flow heterogeneity. The decreased flow heterogeneity along with the increased perfusion rate may be beneficial in terms of O₂ supply in a state of increased cardiac work or hypoxia.

The absence of rheological properties of blood is also involved in decreasing CV under Tyrode perfusion. Actually, CV was still lower in Tyrode- than in blood-reperfused hearts when coronary tone was almost abolished, indicating that the difference of rheological property of perfusates causes the CV difference (Fig. 5). In crystalloid perfusion, the functional capillary density is higher than that in blood perfusion, at least transiently, because of corpuscle-free, low coronary tone, and high-flow conditions (21, 41). The functional capillary density is inversely related to flow heterogeneity (14). The possible increment of capillary diameter under crystalloid perfusion may also reduce the flow heterogeneity as conjectured from the recent in vivo microscopic observation of erythrocyte flows in canine epicardial capillaries, which showed the augmented flow homogenization in capillary beds after the increment of capillary diameter during reactive hyperemia (26). Conversely, under blood perfusion, the higher frequency of microvascular flow interruption due to corpuscles in lower-flow regions will augment the regional flow heterogeneity (16, 41). The contribution of the rheological effect of corpuscles in shaping the heterogeneous flow distribution will increase as the perfusion rate decreases, because erythrocyte aggregation, which can be a leading cause of microvascular flow interruption, increases as a flow shear rate decreases.

The CA difference between Tyrode- and blood-perfused hearts (Fig. 4) indicates that adjacent flows are more similar in blood-perfused hearts. Thus domains comprised of similar high- or low-flow regions appear more clearly under blood perfusion (Fig. 2, middle). Such domains may be placed under

Fig. 7. Representative coronary microangiograms observed at diastole in the same rat left ventricular wall under Krebs perfusion with (A) and without (B) erythrocytes. During the 1-s injection of 0.2 ml of iodine contrast agent (Iopamiron 370; Nihon Schering), the heart was irradiated through a 2-ms shutter with 33-keV X-rays. X-rays passing through the heart were detected with an X-ray SATICON camera (Hamamatsu Photonics) with a 10-bit and 1,024 × 1,024-pixel format. Bar, 400 μm.
unitary control (19) in which the vascular subtrees perfusing those domains would function apparently as unitary flow regulators probably due to segmental flow regulatory mechanisms working in local and hierarchical concert through the well-preserved vascular tone. Actually, when coronary tone was almost abolished, CA decreased largely under blood perfusion (Figs. 4 and 6), implying that nearby regional flows are less correlated with one another under less preserved vascular tone. However, CA was still higher in blood- than in Tyrode-reperfused hearts (Fig. 6). Erythrocyte flows will be also involved in forming domains comprised of similar high- or low-flow regions. In lower-flow domains, erythrocytes aggregation will occur with higher frequency and make the blood more viscous, resulting in the enhancement of the separation of high- and low-flow domains.

**Temporal flow fluctuations.** On the basis of the sequential application of microspheres, early studies demonstrated that a temporal flow difference in a given region was relatively small compared with a spatial flow difference. Thus the distribution of regional myocardial flows has been considered quite stable (25, 37, 45), matching with local energy turnover and gene/protein expression (11, 27, 39). However, at the presently focused microvascular levels, temporal fluctuations of regional flows seem to be comparable in magnitude to spatial flow variations as shown in CV and CA differences between blood-bolus- and blood-60 s-perfused hearts. The flow distributions in blood-bolus-perfused hearts are regarded as “snapshots” of regional flow distribution, whereas in blood-60 s-perfused hearts, the 60-s averaged flow distributions were obtained. The lower CV and the lower CA in blood-60 s-perfused hearts will be attributed probably to the time-averaged effect of quasi-periodic fluctuations of regional flows, which will reduce the range of regional flow variations and prevent the formation of stationary domains of low- or high-flow regions. Microvascular vasomotion, which is likely to exhibit quasiperiodic or chaotic behaviors (4, 42), possibly drives such temporal flow fluctuations. In addition, stochastic flow dynamics of corpuscles in capillary beds or at microvascular bifurcations seem to contribute to temporal flow fluctuations pronouncedly in low-flow regions (14). Averaged for longer time, the difference of spatial flow patterns between blood- and Tyrode-perfused hearts would decrease because of the existence of long-term fluctuations with periods of >60 s (4, 23, 42). In crystalloid-perfused myocardium, temporal flow fluctuations will barely disturb the spatial flow distribution because of the attenuated microvascular tone and the absence of corpuscles as well. Actually, myocardial flow distribution in Tyrode-perfused rabbit hearts remained almost unchanged in our previous study (31).

**Study limitations.** Flow measurements during the infusion of a vasodilator-like adenosine rather than during reactive hyperemia will be most required for understanding the sole effect of corpuscular flows on myocardial flow distributions. In the present experimental setting, however, it is practically impossible for a support rat to maintain the perfusion of an isolated rat heart with maximal vasodilation. The stable blood supply from one support rat will be 5–7 ml·min⁻¹·g⁻¹ at the most, whereas the heart with maximal vasodilation may require much more. Furthermore, when a vasodilator agent is used, it also circulates a support rat and reduces carotid arterial outflow allocated for the isolated heart perfusion. Therefore, in the present study, flow distributions during maximal vasodilation were approximated by those measured during the peak plateau of the reactive hyperemia. Although region-to-region or microvascular site-to-site asynchronous vasodilation during reactive hyperemia (13, 24) prohibits the complete removal of coronary tone effects on regional flows, coronary tone could not be involved in causing the differences of flow distributions under Tyrode and blood perfusion. Future experiments should, however, be designed to elucidate more clearly the role of corpuscular mechanistic factors in the determination of regional flows.

In our previous in vivo studies (29, 30) on rabbit myocardial perfusion, CV and CA increased with depth of myocardium; in the present study, however, neither of them showed a transmural difference even under blood perfusion. The systolic extravascular compressible force, which varies spatially to an exclusively large extent in subendocardium (20), would contribute to forming the transmural difference of within-layer flow distribution. In the present Langendorff heart preparation, however, the LV balloon or the empty LV would modify the myocardial motion, possibly suppressing the subendocardial extravascular force variation. Right ventricular pacing might also disturb the gradient of transmural flow distribution through a modification of regional strains (46, 48).

**Advantage of tracer-digital radiography.** The present tracer-digital radiography has the advantage in evaluating regional flow distribution of a comparatively large tissue area on the scale of 100 μm without capillary and arteriolar obstruction. Although the microsphere method has been sophisticated (7, 32), unavoidable artifacts based on microvascular embolization and biasing of microsphere flow at microvascular bifurcations limit the measurement resolution. Most recently, the fast high-resolution two-dimensional NMR technique for myocardial perfusion imaging in beating hearts was developed to achieve the resolution comparable to the present method (5). This technique will be useful to describe the detailed dynamics of regional flows; at the present time, however, it would be suitable for the measurement under high-flow conditions as in crystalloid-perfused hearts, and some degradation in resolution is inevitable in blood perfusion or low-flow perfusion states.

In conclusion, the spatial patterns of microcirculatory flow distribution differed significantly between rat LVs under Tyrode and blood perfusion. The flow heterogeneity and the local flow similarity were both lower under Tyrode perfusion. During maximal hyperemia, these pattern differences of flow distribution decreased due to the larger vasodilatory response under blood perfusion, however, the differences were still significant. Myocardial performances under Tyrode and blood perfusion were similar, but coronary flow reserve was smaller under Tyrode perfusion. These results indicate that the less preserved coronary tone and the lack of blood corpuscles under Tyrode perfusion are both responsible for producing the different pattern of flow distribution to that under blood perfusion. When the blood flow distribution was averaged for 60 s, the flow heterogeneity and the local flow similarity were significantly lowered, implying that temporal flow fluctuations for short-term periods of 60-s order will function to homogenize regional perfusion.
REFERENCES


