Loss of elastic recoil in postischemic myocardium induces rightward shift of the systolic pressure-volume relationship

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Krams, Rob, Maarten Janssen, Chris Van Der Lee, Jan Van Meegen, Jan Willem De Jong, Cornelis J. Slager, and Pieter D. Verdouw. Loss of elastic recoil in postischemic myocardium induces rightward shift of the systolic pressure-volume relationship. *Am. J. Physiol.* 267 (*Heart Circ. Physiol.* 36): H1557–H1564, 1994.—Ischemia-induced systolic dysfunction has been ascribed to changes in cellular excitation-contraction coupling and diastolic dysfunction because of disruption of the extracellular collagen matrix. Therefore, systolic and diastolic pressure-volume relationships and O_2 consumption were determined before and after 5 min of global ischemia in isolated blood-perfused porcine hearts. The slope of the systolic pressure-volume relationship was 7.2 ± 0.6 (SE) mmHg·ml^{-1}·100 g^{-1} (n = 18) at baseline and did not change during reperfusion, but the systolic volume intercept shifted from 1.0 ± 0.4 ml/100 g at baseline to 3.7 ± 1.4, 4.1 ± 1.1, and 4.2 ± 0.9 ml/100 g at 15, 30, and 60 min of reperfusion, respectively (all $P < 0.05$). The diastolic volume intercept was 8.2 ± 0.7 ml/100 g at baseline and remained unchanged during reperfusion. Therefore, the difference of the systolic-diastolic volume intercepts, an index of elastic recoil forces, was decreased to 57 ± 8, 49 ± 7, and 47 ± 9% of baseline values ($P < 0.05$). The shift of the systolic pressure-volume relationship was accompanied by a transient decrease of contractile efficiency (slope of O_2 consumption-pressure-volume-area relationship) at 15 min of reperfusion (from 43 ± 6 to 27 ± 7%). We hypothesize that the rightward shift of the systolic pressure-volume relationship was compatible with a decrease of elastic-restoring forces, probably induced by alterations in the extracellular collagen matrix and/or the cytoskeleton, and thereby our data imply that left ventricular dysfunction of postischemic myocardium does not result solely from disturbances in excitation-contraction coupling.

isolated pig hearts; myocardial ischemia and reperfusion; time-varying elastance; pig

REVERSIBLE CARDIAC dysfunction induced by brief periods of ischemia consists of a systolic and a diastolic component (3, 11, 15, 16, 18, 37, 38). Whereas the decrease in systolic function has been related to disturbances in cellular excitation-contraction (EC) coupling (16, 18), alterations in the extracellular collagen matrix may be involved in diastolic dilation (3, 37). It has been postulated that the collagen matrix, however, not only prevents diastolic dilation but also plays a role in the twisting motion of the heart (23), the negative cavity pressures at low ventricular volumes (diastolic suction) (36), and the coordination of force production as well as the transmission of force between muscle fibers (7, 9). These phenomena are ultimately related to systolic function. For instance, subatmospheric intraventricular pressures measured during early diastole at low intraventricular volumes in open-thorax dogs (diastolic suction) have been postulated to be caused by the release of energy stored in the extracellular collagen fibers passively stretched during the preceding systole (31). This implies that part of systolic work is used to passively stretch these collagen fibers, and impairment of the function of the collagen matrix affects systolic and diastolic myocardial function.

According to the time-varying elastance concept (26), the slope of the systolic pressure-volume relationship (SPVR) or E_{max} reflects myocardial contractility, which is a function of cellular EC coupling. Originally the zero pressure-volume intercept of the SPVR was assumed to be identical to the zero pressure-volume intercept of the diastolic pressure-volume relationship (DPVR), stressing the time-varying nature of the slope or elastance (26). Recently, it has been recognized that the systolic and diastolic zero pressure-volume intercepts are not identical (32, 38). The difference between the systolic and diastolic zero pressure-volume intercepts induces a range of left ventricular volumes where diastolic pressure is subatmospheric [diastolic suction (36)]. Because the subatmospheric pressure is related to the actual diastolic volume, the difference between the systolic and diastolic volume intercepts has been proposed as an index of the elastic recoil forces that underlie the diastolic suction (30, 32). Although the effect of different interventions on this index of elastic recoil forces has been studied, the accompanying effect on the position and slope of the entire SPVR has not yet been evaluated (32).

We reasoned that a pure reduction in elastic recoil forces induced by alteration in the function of the extracellular collagen matrix of postischemic myocardium (3, 38) might lead to a rightward shift of the SPVR toward the DPVR and consequently a decrease of the elastic recoil index without a change in its E_{max}. In contrast, a pure disturbance of the cellular EC coupling would induce a change in contractility and consequently a change in E_{max} without a large change in the systolic zero pressure-volume intercept and elastic recoil index.

The aim of the present report therefore is to evaluate systolic and diastolic left ventricular function in terms of pressure-volume relationships before and after 5 min of ischemia and the relative contributions of each above-mentioned mechanism on systolic function. This short period of ischemia was chosen to avoid induction of myocardial necrosis.

Because it has also been postulated that the collagen matrix coordinates the systolic and diastolic force generation between fibers (7, 9), an altered function of the extracellular collagen matrix might also induce a less efficiently working myocardium. We therefore also evalu-

ated the energy transformation of the myocardium by measuring the relationship between O_2 consumption and the total mechanical work or the pressure-volume-area (PVA) (26).

MATERIALS AND METHODS

General

All experiments were performed in accordance with the "Guiding Principles in the Care and Use of Animals," as approved by the Council of the American Physiological Society, and under the regulation of the Committee on Animal Experimentation of the Erasmus University Rotterdam. Hearts were obtained from overnight-fasted cross-bred Landrace Yorkshire pigs (10–21 kg) that had been anesthetized as described earlier (15).

Isolation Procedure

After exposure of the heart via a midsternal sternotomy, the blood of the pig was exchanged with a dextran solution (Isodex, NPBI, Emmer Compascuum, The Netherlands). Depending on the weight of the animals, 0.5–1 liter of whole blood could be collected in standard blood bags (Compoflex, NPBI). The heart was then fibrillated by placing a battery on the free wall, and the aorta was clamped and incised distally from the clamp to prevent air from flowing into the coronary vascular bed. The heart was excised and arrested in 500 ml of St. Thomas' Hospital cardioplegic solution at 4°C. All further surgical procedures were done in this solution and at this temperature. The whole process from excision to the restoration of coronary flow, including surgical preparation (see below), required < 30 min.

Isolated Heart Model

The experimental model consists of a bubble oxygenator (Polystan, Copenhagen, Denmark), a double-lumen reservoir, an occlusive roller pump (Verder, Vleuten, The Netherlands), and a double-lumen windkessel to dampen pressure oscillations. The pump was pressure controlled with a feedback system developed at our workshop. The windkessel and reservoir served as heat exchangers (37°C). A blood filter (40 μm; Pall Biomedical, Portsmouth, UK) was inserted proximal to the windkessel to prevent thrombocyte aggregates from reaching the heart.

Before the heart was isolated, the system was primed with a bovine albumin solution (10 g/l; fraction V, Sigma Chemical, St. Louis, MO) for 1 h. Pilot studies (n = 3) revealed that this approach prevented platelet aggregation for up to 3 h, as assessed by platelet count and (relative) platelet distribution. After the system was primed, it was filled with 500 ml of the gelatin-derived blood plasma substitute Haemaccel (Behringwerke, Hamburg, Germany) to which 10 mM glucose (Merck, Darmstadt, Germany) was added. During the isolation and preparation of the heart, the perfusion system was further filled with blood collected from the same animal to 0.5–1 liter (see above). Preparation of the heart consisted of cannulation of the aorta, the pulmonary artery, and the apex. The aorta was cannulated with a metal cannula that could easily be connected to the windkessel. The pulmonary artery was cannulated, and the cannula was connected to the oxygenator to ensure that blood did not contact air. The left atrium was incised, the mitral valve and chordae tendineae were carefully dissected, and the apex was cannulated with a stiff cannula (1 mm OD) to collect Thebesian outflow. A purse-string suture was placed around the left atrium. Then a latex balloon (unstressed volume 80 ml) was inserted into the left ventricle via the mitral valve to allow isovolumic left ventricular pressure generation independent of coronary perfusion pressure. The balloon was mounted on a sturdy transparent Perspex frame to detect (small) air bubbles. An adjustable rim could be connected to the frame just above the connection with the balloon to adjust for variable mitral openings and therefore ensure closure of the left ventricle. A glass cannula was inserted from the top of the frame into the balloon to measure pressure inside the left ventricular cavity. Tight coupling between the balloon and the endocardium could be achieved by creating low pressures outside the balloon through the apex cannula. Two pacing wires were sewed on the conus arteriosus and connected to a pacemaker to control heart rate. Total coronary flow was determined by the speed of the roller pump, which was calibrated for each experiment.

Arterial and Venous Contents of O_2, Lactate, and Purine

Arterial blood samples, withdrawn 2 cm above the coronary ostia, and coronary venous blood samples, withdrawn via the pulmonary artery cannula, were collected to measure P_{O_2}, O_2 saturation (S_{O_2}), and hemoglobin (model OSM2, Radiometer, Copenhagen, Denmark). These data were converted to arterial and venous O_2 content (C_{O_2}) by application of the following formula: $C_{O_2} = Hb * S_{O_2} + 0.00138 * P_{O_2}$, where Hb is hemoglobin concentration (mmol/l). Arterial and coronary venous blood samples were also analyzed for total purine content, which is the sum of adenosine, inosine, hypoxanthine, xanthine, and urate, by use of high-performance liquid chromatography (28), and lactate content, determined with the Elan analyzer (Merck). The difference between the arterial and venous O_2, purine, and lactate contents was multiplied by coronary flow to obtain the myocardial consumption of each parameter.

Experimental Protocols

Stability of preparation (control series, n = 6). The stability of the preparation was evaluated by determining SPVRs at different time points during 3 h of perfusion. The volume of the intraventricular balloon was changed over a wide range of values, and at each volume the resulting left ventricular pressure was recorded while perfusion pressure was kept at a constant level of 100 mmHg. Between the determination of SPVRs, left ventricular volume was set to create a systolic pressure of ~90 mmHg. Heart rate was kept constant by atrial pacing at 100–120 beats/min. Arterial and coronary venous blood samples were collected for the measurement of ATP catabolism (purine efflux; see above).

Ischemia-reperfusion protocol (intervention series, n = 18). Global ischemia was induced by stopping total coronary inflow for 5 min, then the clamp was released and the coronary perfusion pressure and flow were restored. Left ventricular pressure-volume and myocardial O_2 consumption ($M\dot{V}O_2$) were measured again when peak systolic pressure reached a new steady state. Because the $M\dot{V}O_2$ measurements for one O_2 consumption-PVA (see below) relationship lasted 45 min, we determined only two $M\dot{V}O_2$-PVA relationships during the reperfusion phase in each heart: at 15 and 30 min of reperfusion in 8 hearts (*group I*) and at 30 and 60 min of reperfusion in 10 other hearts (*group II*). To relate the $M\dot{V}O_2$ per beat (ml O_2/beat) to energy conversion (mJ/beat), aerobic conditions are necessary. This assumption was tested by determining lactate production during baseline conditions and at 1, 5, 10, 15, 30, and 60 min of reperfusion. Viability of myocardial tissue was evaluated using the triphenyltetrazolium chloride staining technique (14). At the end of each experiment, the hearts were blotted and extracardiac fat and large arteries were removed and weighed.

Data Analysis and Statistics

Because left ventricular volume was kept constant between the determination of the SPVRs, the degree of left ventricular dysfunction due to the ischemic period could be evaluated as *1*) $(P_{sys,st}/P_{sys,bl}) * 100$ and *2*) $(devP_{st}/devP_{bl}) * 100$, where P_{sys} and devP are peak systolic and developed left ventricular pressure, the subscript bl represents values obtained at baseline just before the start of the ischemic period, and the subscript st indicates values obtained during steady-state conditions in the reperfusion phase just before the determination of the second SPVR. SPVR and DPVR were constructed by measuring peak systolic and minimal diastolic left ventricular pressures at each known balloon volume. Regression analysis was applied to the systolic data to calculate E_{max} (i.e., slope of the regression line) and the systolic zero pressure-volume intercept ($V_{s,0}$). A linear and a quadratic fit were applied to these systolic pressure-volume relationships (2). The quadratic model was considered superior to the linear model when the F statistic $[(SS_2 - SS_1)/MS_2$, where SS is sum of squares, MS is mean sum of squares, and subscripts 1 and 2 represent 1st- and 2nd-order models, respectively] reached levels of statistical significance ($P < 0.05$). When the pressure-volume relationships are linear, the slopes are independent of volume. In case of a nonlinear relationship, the slopes at zero transmural pressure were calculated (2). The same procedure was applied to the diastolic pressure-volume points to obtain the minimal diastolic elastance (E_{min}) and diastolic zero pressure-volume intercept ($V_{d,0}$, ml). The ratio $(V_{d,0} - V_{s,0})/V_{d,0}$ was calculated as an index of elastic recoil forces (32).

In addition, the volume shift of the SPVR at a left ventricular pressure of 50 mmHg was calculated from the coefficients of the regression equations. Because the change of $V_{s,0}$ could be interpreted as a pure shift of the SPVR, the remaining volume shift at 50 mmHg is due to an additional rotation of the SPVR or change of E_{max}. The PVA was calculated from the area enclosed by the diastolic and systolic PVA at a given left ventricular volume (26).

$M\dot{V}O_2$ was divided by heart rate to obtain $M\dot{V}O_2$/beat and subsequently normalized to 100 g of tissue (ml $O_2 \cdot beat^{-1} \cdot 100\ g^{-1}$) and converted to $mJ \cdot beat^{-1} \cdot 100\ g^{-1}$ (26). The inverse of the slope of the O_2-PVA relationship is a measure of the contractile efficiency (26). Arteriovenous concentration differences for purine and lactate were calculated and multiplied by coronary flow to calculate purine and lactate production or consumption. Data are presented as means ± SE. Statistical analysis was performed for each variable by repeated-measures analysis of variance. When significance was reached ($P < 0.05$), paired t-tests were applied with a Bonferroni correction for multiple measurements. Individual values were tested with a paired t-test.

RESULTS

Control Series

The end-systolic pressure-volume relationships did not change over the perfusion time of 210 min, as shown by an unchanged E_{max} and $V_{s,0}$ (Table 1). In addition, the slope and volume intercept of the DPVR did not change over time (Table 1). Diastolic zero pressure-volume intercepts (8.9 ± 2.1 ml/100 g at baseline) were much higher than the systolic zero pressure-volume intercepts and did not change over time (Table 1).

Purine efflux was 3.8 ± 2.8 $nmol \cdot min^{-1} \cdot g$ wet wt^{-1}. With an ATP concentration of 20 μmol/g wet wt (6), this implies that ATP breakdown was 4 ± 1%, which is negligible. Thus, on the basis of these measurements, we concluded that the preparation remained stable up to 3.5 h of perfusion. This period exceeded the duration of the ischemia-reperfusion protocol used in the next series of experiments (see below).

Table 1. *Slopes and volume intercepts of end-systolic and end-diastolic pressure-volume relationships in blood-perfused isolated pig hearts without interventions (control series)*

	Perfusion Time, min			
	30	90	150	210
E_{max}, $mmHg \cdot ml^{-1} \cdot 100\ g^{-1}$	4.9 ± 0.8	5.1 ± 0.8	5.3 ± 0.6	4.6 ± 1.0
$V_{s,0}$, ml/100 g	2.5 ± 1.8	1.9 ± 1.5	2.8 ± 1.5	2.2 ± 1.5
E_{min}, $mmHg \cdot ml^{-1} \cdot 100\ g^{-1}$	1.2 ± 0.7	0.9 ± 0.6	1.3 ± 0.8	1.3 ± 0.7
$V_{d,0}$, ml/100 g	8.9 ± 2.1	11.9 ± 2.2	10.1 ± 1.4	8.6 ± 1.6

Values are means ± SE of 6 pigs. E_{max} and E_{min}, slopes of systolic and diastolic pressure-volume relationships, respectively; $V_{s,0}$ and $V_{d,0}$, zero systolic and diastolic pressure-volume intercepts, respectively.

Myocardial Mechanics

Figure 1 exemplifies the generation of the pressure-volume relationships before and after 5 min of ischemia and after 15 min of reperfusion. After a small overshoot during the very early reperfusion period, left ventricular systolic pressure and left ventricular developed pressure declined to ~70 and 60% of baseline, when preload was kept constant at 10 ml (Fig. 1). These last values were very similar for all experiments (66 ± 5 and 59 ± 4%, respectively, $P < 0.05$). This decrease in left ventricular pressure was not due to a rotation but to a rightward shift of the entire SPVR, whereas DPVRs rotated (Fig. 2). For all the experiments combined, the slope of the SPVR or E_{max} values did not change statistically, except at 30 min of reperfusion in *group II* ($P < 0.05$ vs. baseline; Table 2). The systolic zero pressure-volume intercepts, however, were increased at every time point during the reperfusion phase ($P < 0.05$ vs. baseline; Table 2). There was no significant change between baseline values of *groups I* and *II*. The DPVRs showed the opposite effect: a significant increase of the slope and no change in the diastolic zero pressure-volume intercepts (Table 2). When the relative changes in E_{max} $(\Delta E_{max}/E_{max,baseline})$ and the difference in systolic and diastolic zero pressure-volume intercepts normalized for baseline values $(V_{d,0} - V_{s,0}/V_{d,0,baseline})$ were plotted vs. reperfusion time (Fig. 3), no significant changes in E_{max} were found, except at 30 min of reperfusion for *group II*, whereas an early significant decrease in the difference of the volume intercepts was measured.

The shift of the SPVR calculated at a left ventricular pressure of 50 mmHg consisted of 80 ± 4, 82 ± 3, and 89 ± 4% of a translation at 15, 30, and 60 min, respectively, of reperfusion (for details see MATERIALS AND METHODS), while the remainder was caused by a rotation of the SPVR caused by a change of E_{max}.

To further exclude a cellular contractility-related mechanism underlying the observed rightward shift of the SPVR, three additional experiments were performed. In one heart, a known negative inotropic agent

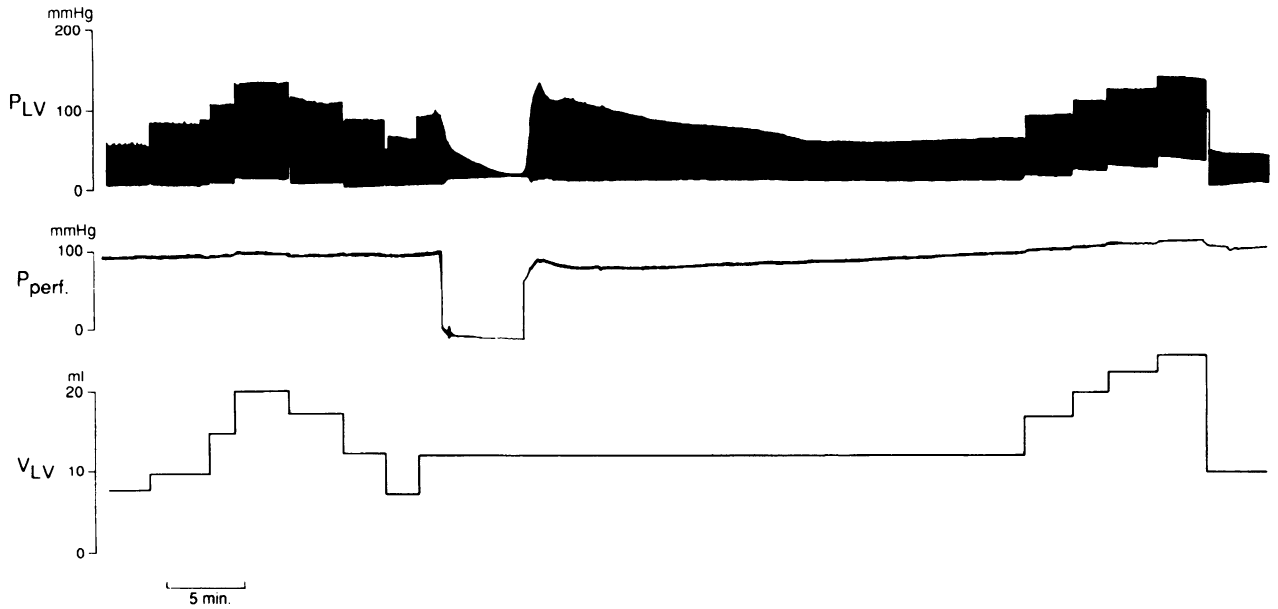

Fig. 1. Representative examples of left ventricular pressure (P_{LV}, mmHg), perfusion pressure (P_{perf}, mmHg), and left ventricular volume (V_{LV}, ml) before and after 5 min of ischemia.

(lidocaine, 40 mg) was infused during baseline conditions. This decreased E_{max} by 39%, while the $V_{s,0}$ remained unchanged. In two other hearts, Ca^{2+} (3 meq) and lidocaine (40 mg) were infused at 60 and 120 min, respectively, of reperfusion, i.e., when changes in E_{max} and $V_{s,0}$ were not observed (Fig. 3). Ca^{2+} increased E_{max} by 30% (from 3.0 to 3.9 mmHg/ml) compared with 30 min of reperfusion. Lidocaine decreased E_{max} by 30% (from 3.3 to 2.0 mmHg/ml) compared with 60 min of reperfusion. $V_{s,0}$ remained unchanged in both experiments.

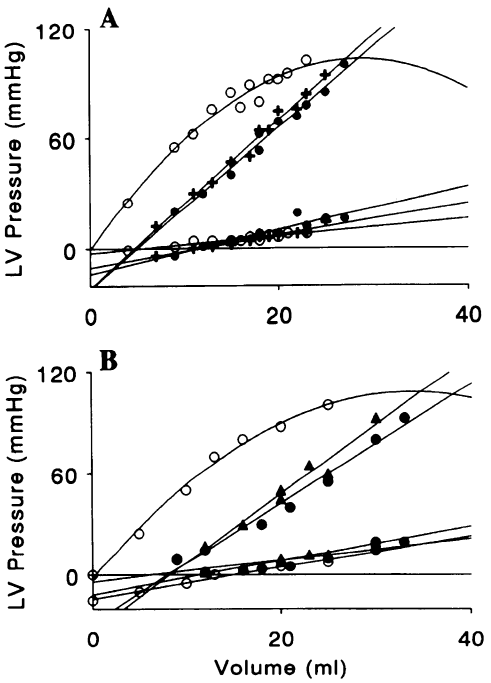

Fig. 2. Representative examples of left ventricular (LV) pressure-volume relationships from *group I* (*A*) and *group II* (*B*) at baseline (○) and 15 (+), 30 (●), and 60 min (▲) of reperfusion.

Myocardial Energetics

Examples of an $M\dot{V}O_2$-PVA relationship for *groups I* and *II* are presented in Fig. 4. These graphs and the average response of all experiments (Table 2) indicate that the inverse of the slope (contractile efficiency) of the

Table 2. *Myocardial mechanics and energetics of blood-perfused isolated pig hearts during reperfusion after 5 min of global ischemia*

	Baseline	Reperfusion Time, min	
		15	30
Group I (n = 8)			
E_{max}, mmHg·ml^{-1}·100 g^{-1}	7.1 ± 1.0	5.4 ± 0.9	5.6 ± 0.8
$V_{s,0}$, ml/100 g	0.8 ± 0.8	3.7 ± 1.4*	4.7 ± 1.9*
E_{min}, mmHg·ml^{-1}·100 g^{-1}	1.2 ± 0.3	1.7 ± 0.4*	1.4 ± 0.4*
$V_{d,0}$, ml/100 g	7.3 ± 0.7	7.9 ± 1.1	7.8 ± 1.0
Coronary flow, ml·min^{-1}·100 g^{-1}	186 ± 25	197 ± 37	135 ± 37
Efficiency, %	43 ± 6	27 ± 7*	40 ± 6
Unloaded $M\dot{V}O_2$, mJ·beat^{-1}·100 g^{-1}	566 ± 159	434 ± 103	369 ± 121
	Baseline	Reperfusion Time, min	
		30	60
Group II (n = 10)			
E_{max}, mmHg·ml^{-1}·100 g^{-1}	7.5 ± 0.8	5.4 ± 0.7*	5.6 ± 0.8
$V_{s,0}$, ml/100 g	1.2 ± 0.4	3.5 ± 1.1*	4.2 ± 0.9*
E_{min}, mmHg·ml^{-1}·100 g^{-1}	1.1 ± 0.2	1.7 ± 0.4*	1.9 ± 0.4*
$V_{d,0}$, ml/100 g	8.7 ± 1.4	9.2 ± 1.2	10.2 ± 1.6
Coronary flow, ml·min^{-1}·100 g^{-1}	183 ± 30	194 ± 40	163 ± 40
Efficiency, %	43 ± 8	41 ± 8	41 ± 10
Unloaded $M\dot{V}O_2$, mJ·beat^{-1}·100 g^{-1}	735 ± 125	561 ± 110	457 ± 86*

Values are means ± SE. Efficiency, slope of O_2 consumption-pressure-volume-area relationships; unloaded O_2 consumption ($M\dot{V}O_2$), intercept at zero pressure-volume-area. *$P < 0.05$ vs. baseline.

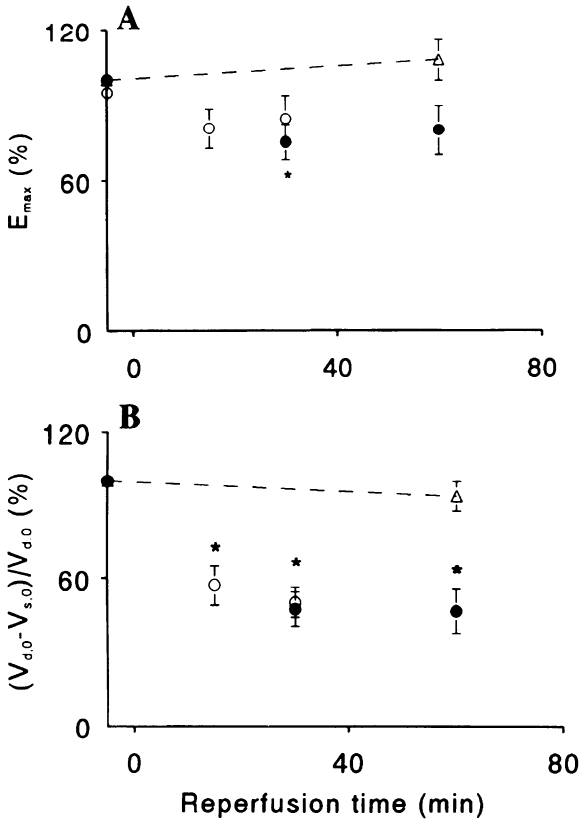

Fig. 3. Relative changes of slope of systolic pressure-volume relationship (E_{max}; ΔE_{max}, reperfusion normalized for $E_{max,baseline}$) and difference of systolic ($V_{s,0}$) and diastolic ($V_{d,0}$) zero pressure-volume intercepts normalized for its value at baseline for *group I* (○) and *group II* (●) vs. reperfusion period. Leftmost data points represent normalized baseline values. △, Control group. *$P < 0.05$ vs. baseline.

$M\dot{V}O_2$-PVA relationship decreased transiently at 15 min of reperfusion (from 43 ± 6 to 27 ± 7%) but returned to baseline values at 30 and 60 min of reperfusion (Table 2). The unloaded O_2 consumption (unloaded $M\dot{V}O_2$) showed a decline during the reperfusion period. Significance was reached at 60 min of reperfusion ($P < 0.05$ vs. baseline; Table 2). No significant differences between baseline values and 30 min of reperfusion were found between the groups. Relative changes for both groups combined are presented in Fig. 5. Contractile efficiency was decreased by 35% at 15 min ($P < 0.05$ vs. baseline) but returned to 94 and 95% of baseline values at 30 and 60 min of reperfusion, respectively (P = NS). The unloaded O_2 consumption decreased steadily to 77, 71, and 62% at 15, 30, and 60 min, respectively. Significance was reached at 60 min of reperfusion ($P < 0.05$).

At baseline the myocardium slightly consumed lactate (0.34 ± 0.26 $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1}), but this was changed to production during the very early perfusion period (−3.1 ± 0.5 and −0.6 ± 0.1 $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1} at 1 and 10 min of reperfusion, respectively, both $P < 0.05$ vs. zero) and returned to baseline values (i.e., consumption) at 15 min of reperfusion (1.1 ± 1.0 $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1}). There were no further changes during the remainder of the reperfusion period.

Viability of Myocardial Tissue

None of the three hearts that were stained with triphenyltetrazolium chloride at the end of the protocol showed signs of necrosis.

DISCUSSION

The aim of the present study was to measure the changes in the SPVR and energy conversion of the left ventricle, under well-defined conditions, in the early reperfusion phase after a brief period of ischemia. A 5-min period of ischemia was chosen to avoid tissue necrosis, which was confirmed by the triphenyltetrazolium chloride staining technique (14). From these data, we conclude that we were studying viable myocardial tissue.

Mechanical Response to a Brief Period of Ischemia

The main observation in this study is that the observed decrease in left ventricular systolic pressure and left ventricular developed pressure is predominantly due to a rightward shift of the entire SPVR in the (early) reperfusion phase (~80%) and a relative small decrease of contractility as indexed by the E_{max}. Because we did not observe a rightward shift of the SPVR over the same perfusion period in the control series, this rightward shift of the SPVR in the postischemic period is the consequence of the preceding ischemic period. Furthermore, infusion of known inotropic agents (this study; 2, 26), in normal and postischemic myocardium (this study),

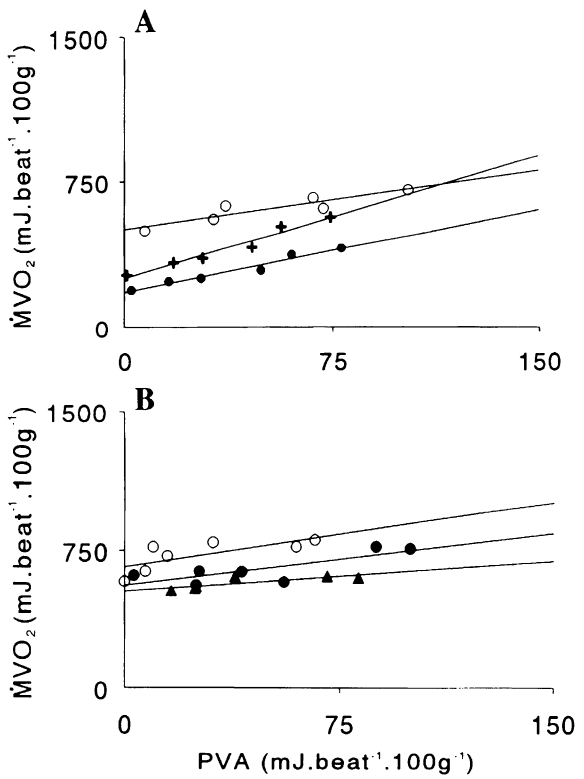

Fig. 4. Mocardial O_2 consumption ($M\dot{V}O_2$)-pressure-volume-area (PVA) relationship of *group I* (*A*; ○, baseline; +, 15 min of reperfusion; ●, 30 min of reperfusion) and *group II* (*B*; ○, baseline; ●, 30 min of reperfusion; ▲, 60 min of reperfusion).

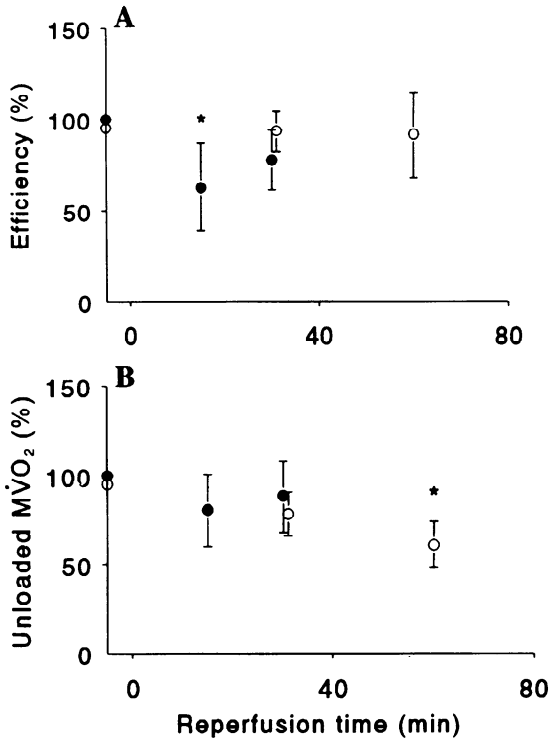

Fig. 5. Relative changes of myocardial efficiency (*A*) and unloaded O_2 consumption (*B*) for *group I* (●) and *group II* (○) combined. *$P < 0.05$ vs. baseline.

induces changes in E_{max} without an important effect on the systolic zero pressure-volume intercept. This implies that the rightward shift of the SPVR might not be related to a change of contractility and suggests a non-EC coupling-related origin of the underlying mechanism (see below).

A shift of the global SPVR, without a change in the slope (E_{max}), has also been measured in regionally ischemic human and canine hearts and isolated postischemic hearts (24, 30, 33, 34). The data on regional ischemia have been interpreted with the aid of a compartment model employing a normal and an ischemic elastance (33). To fully describe the changes of the global pressure-volume relationships, the SPVR under regionally ischemic conditions had to be shifted rightward with respect to the control region (constant regional elastance). This assumption has been confirmed by measurements of the elastance of the ischemic region (8). Although these observations were made on regionally postischemic ventricles, where in the ischemic region bulging occurs and the remote myocardium partially compensates for the ischemic region, these data indirectly support our observations. Interestingly a loss of diastolic suction has been measured in patients during ischemia (4, 5), indicating that rightward shifts of the SPVR measured in humans may be accompanied by a loss of diastolic suction, as found in our isolated heart study. The above-mentioned findings of a parallel shift of the SPVR (constant E_{max}) have generally been interpreted as a decrease in contractility (8, 24, 30, 33). Theoretically this violates the original interpretation of the time-varying elastance concept, where changes in E_{max} were believed to reflect changes of contractility (26, 30). Our data indicate, at least for the globally postischemic myocardium, that a change of E_{max} still reflects a change of contractility, whereas a shift of the SPVR might be related to a different mechanism (see below).

Energetic Response to a Brief Period of Ischemia

Because we added Haemaccel, which reduces hemaglobin content and consequently the arterial O_2 content, to the blood, it could be argued that the preparation was hypoxic not only during ischemia but also at baseline and during reperfusion. However, three observations argue against such reasoning: *1*) ATP breakdown, estimated by purine efflux (6), was very small during the same perfusion period in our control series; *2*) the hearts consumed lactate before the ischemic period in our intervention series, indicating the absence of anaerobic metabolism; and *3*) unloaded O_2 consumption and myocardial efficiency during baseline conditions were similar in donor-perfused isolated hearts and closed-chest animals (29, 30).

Possible explanations for the decrease in myocardial efficiency at 15 min of reperfusion are *1*) a change of substrate consumption during the early reperfusion phase (27) and *2*) an intrinsic change within the myocardial wall. The first explanation seems unlikely, because we added extra glucose (final concn 10 mM) to the blood to minimize effects of changes in substrate consumption. If, however, a (small) change in substrate consumption still occurred, it has been reported to change to relatively more glucose consumption (27, 35). Because of a slightly higher phosphorus-to-O_2 ratio with glucose combustion, a slight increase in myocardial efficiency is expected, and this might therefore even underestimate the observed decrease in myocardial efficiency in the early reperfusion phase. We therefore conclude that the decrease in myocardial efficiency reflects a change of an intrinsic myocardial wall property. Although it has also been postulated that the extracellular collagen matrix serves to optimize force transmission between fibers (7, 9) and an altered function of the collagen matrix, therefore, might induce a less efficiently working myocardium, the restoration of contractile efficiency when the rightward shift of the SPVR was still apparent makes this explanation less likely. These observations might implicate the generation of futile cycles of ATP or a less efficient ATP-myofibrillar system (30).

An unchanged and a decreased contractile efficiency have been reported in postischemic myocardium (1, 17, 21, 24). In all these studies, the ischemic period was longer than that in the present study, inducing a disturbance of cellular EC coupling as indexed by a decrease of E_{max} (1, 21), making the comparison between these and our studies difficult.

Underlying Mechanism

It has recently been shown that an abundant collagen network exists between (groups of) myocytes (38). In addition, connections exist among this collagen network, the cytoskeleton, and proteins inside the myocyte that anchor the contractile proteins to the sarcolemma (12, 22). The precise function of these structures is presently unknown, but it has been postulated that they

play an important role in diastolic suction and the twisting motion of the myocardium (23, 36), which enables these structures to modulate the coupling between diastolic and systolic function (23, 26, 36). To account for these observations, the original version of the time-varying elastance model has been expanded (26, 32), and this modified model now predicts subatmospheric left ventricular pressures at low diastolic left ventricular volumes and a difference between the systolic and diastolic zero pressure-volume relationships (26, 32). The latter prediction has been used as an index for the forces that underlie the diastolic suction (elastic recoil) (26, 32).

The main finding of the present study is the predominant parallel shift of the SPVR in postischemic myocardium. A negative inotropic intervention of the preischemic left ventricle rotated the SPVR clockwise, comparable to other reports (26, 32). In addition, negative and positive inotropic interventions in the postischemic myocardium also only affected the slope of the SPVR. This indicates a non-EC-coupling-related mechanism underlying the shift of the SPVR. The close relationship between the shift of the SPVR and the index of elastic recoil forces indicates a disturbance of the function of the extracellular collagen matrix and/or the cytoskeleton. Indeed it has been shown that during ischemia the cytoskeleton and the extracellular collagen matrix become damaged, leading to the formation of extracellular and cellular edema during the postischemic reperfusion phase (25, 38), in accordance with the increased diastolic stiffness found in this study (Table 2). Furthermore, it has been shown by the same authors, in open-thorax animals, that related to the disturbed function of the extracellular collagen matrix the diastolic sarcomere length of the myocytes in the stunned region is increased with respect to the control region (37, 38). If these observations are applicable to our studies, it might imply that, despite the increased diastolic stiffness, diastolic sarcomere lengths are increased at similar left ventricular volumes with respect to baseline values. The resulting rightward shift of the diastolic pressure-sarcomere relationship in those studies (37, 38) could provide an explanation for the parallel shift of the SPVR.

Cellular edema in the postischemic myocardium also increases the spacing between the myofilaments (37), which has been shown to decrease force development of skinned fibers at similar calcium concentrations (10, 12, 19, 20). These observations might therefore alternatively explain the parallel shift of the SPVR in postischemic myocardium at similar DPVRs. The latter observations, however, might also explain why changes in E_{max} could still be induced by inotropic interventions in the postischemic myocardium. Because no detailed information is available at the cellular level, we cannot discriminate between the relative contributions of both mechanisms to the resulting left ventricular dysfunction in the present study. Our data suggest, however, that postischemic myocardial dysfunction is not related solely to disturbances of EC coupling (16–18) but also to an altered function of the extracellular collagen matrix and/or the cytoskeleton.

Limitations of the Study

A potential problem in interpreting our results could be the uncoupling of the balloon from the left ventricular endocardial wall (31). However, this does, most likely, not affect our results, inasmuch as the shift of the SPVR occurred over the entire range of balloon volumes studied, while uncoupling of the intraventricular balloon from the endocardial layers is believed to occur only at low balloon volumes (32). Furthermore, Suga et al. (32) evaluated the left ventricular volume changes around zero transmural pressure without the confounding effects of the left ventricular balloon and found a difference between the systolic and diastolic volume intercepts of 7.5 ± 2.5 ml/100 g, which was similar to our measurements during baseline conditions (7.1 ± 0.8 ml/100 g).

O_2 consumption of the whole isolated pig heart is affected by the right ventricle (20). Because we applied suction to the right ventricle, its intraventricular volume was low and independent of the volume changes applied to the left ventricle. This implies that we overestimated the left ventricular unloaded O_2 consumption; i.e., the "real" left ventricular $M\dot{V}O_2$-PVA relationship has to be shifted downward in a parallel fashion. Because after ischemia we stunned the right and the left ventricle, probably, to a different unknown degree, a correction factor, based on a weight criterion, was not useful in our experiments. This could theoretically explain the variability in the $M\dot{V}O_2$-PVA relationships as well as the slightly higher unloaded O_2 consumption found in this study. It cannot, however, be the cause of the change in contractile efficiency.

Although we applied cardioplegia to these hearts to minimize O_2 consumption during the surgical preparation period, this period still lasted up to 30 min, which might have induced oxidative stress at the start of the perfusion period. However, our baseline contractile states are normal to high compared with the other isolated heart and intact animal studies (26, 29, 30). In addition, no change of the SPVRs in the control series was found, which implies that the changes of the SPVR in the intervention group are due to the ischemic period and not to the duration of the preparation period.

In conclusion, we studied pressure-volume relationships sequentially in the reperfused myocardium after a brief period (5 min) of global ischemia. The data were analyzed in terms of the time-varying elastance concept (26, 30). The functional changes of the reperfused myocardium could be characterized by a predominantly rightward shift of the entire peak SPVRs without a large effect on contractility while the diastolic zero pressure-volume intercept relationships remained unaffected. In concert with these measurements was the observation that contractile efficiency was decreased transiently during the early reperfusion phase, while the unloaded O_2 consumption gradually decreased. We forwarded the hypothesis that the observation is related to a defect in elastic restoring forces, probably related to an altered function of the extracellular collagen matrix or intracellular proteins that anchor the contractile proteins to the sarcolemma. Our data imply that the left ventricular

dysfunction of postischemic myocardium does not result solely from disturbances in EC coupling (16–18).

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