J Mol Cell Cardiol **18**, 1207–1218 (1986)

Post-ischemic Cardiac Chamber Stiffness and Coronary Vasomotion: the Role of Edema and Effects of Dextran

W. Mark Vogel, Adam W. Cerel, and Carl. S. Apstein

From the Cardiac Muscle Research Laboratory. Housman Medical Research Center, Departments of Pharmacology and Medicine, Boston University School of Medicine; and the Cardiology Section of the Thorndike Memorial Laboratory, Boston City Hospital, Boston, MA 02118, USA

(Received 6 June 1985, accepted in revised form 24 June 1986)

W. M. Vogel, A. W. Cerel and C. S. Apstein. Post-ischemic Cardiac Chamber Stiffness and Coronary Vasomotion: The Role of Edema and Effects of Dextran. *Journal of Molecular and Cellular Cardiology* (1986) **18**, 1207–1218. Contributions of edema to left ventricular (LV) chamber stiffness and coronary resistance after ischemia were studied in isolated buffer-perfused rabbit hearts, with constant LV chamber volume, subjected to 30 min global ischemia and 60 min reperfusion. During reperfusion hearts were perfused with standard buffer or with 3% dextran to increase oncotic pressure and decrease water content. LV chamber volume was adjusted to an initial diastolic pressure (LVEDP) of 10 mmHg. In nonischemic hearts ($n = 6$) LVEDP was 11 ± 0.3 mmHg and water content was 5.0 ± 0.1 ml/g dry weight after 90 min of perfusion. In untreated ischemic hearts ($n = 8$) LVEDP was 51 ± 4 mmHg and water content was 6.0 ± 0.1 ml/g dry weight after 60 min reperfusion ($P < 0.001$ v. nonischemic). In dextran-treated ischemic hearts ($n = 8$) LVEDP was 38 ± 3 mmHg ($P < 0.05$ v. untreated ischemic) and water content was 5.2 ± 0.1 ml/g dry weight ($P < 0.001$ v. untreated ischemic). Coronary resistance in untreated ischemic hearts increased by 26% from 2.0 ± 0.06 to 2.6 ± 0.06 mmHg/ml/min after 60 min reperfusion. In treated hearts coronary resistance increased by 16% from 1.9 ± 0.09 to 2.2 ± 0.09 mm/Hg/ml/min ($P < 0.01$ v. untreated ischemic). To determine whether the decrease in coronary resistance with dextran could be ascribed to active vasodilation, dilator responses to 2 min hypoxia or 10^{-4} M adenosine were tested in nonischemic and reperfused ischemic hearts. Dilator responses were stable in nonischemic hearts or hearts reperfused after 15 min ischemia but after 30 min ischemia the dilator response to hypoxia was reduced by 72% ($P < 0.025$) and the dilator response to adenosine was eliminated ($P < 0.02$). Thus the response to dextran was unlike that of a direct vasodilator. These data suggest that myocardial edema plays a significant role in maintaining increased ventricular chamber stiffness and coronary resistance during reperfusion after ischemia.

Key Words: Ischemia; Reperfusion injury; Diastolic compliance; Coronary arterial resistance; Myocardial edema; Oncotic pressure; Coronary vasodilator reserve.

Introduction

Reperfusion after prolonged myocardial ischemia or hypoxia may be associated with increased cardiac wall stiffness [*1, 4, 12, 25, 36*] and increased coronary arterial resistance [*1, 7, 18, 23, 41*]. The myocardial edema, which occurs during reperfusion after ischemia [*8, 9, 12, 17, 20, 26*], may play an important role in maintaining this increased cardiac stiffness and increased coronary resistance. Pharmacological interventions, which reduced the degree of myocardial edema induced by ischemia and reperfusion, also decreased coronary arterial resistance [*8, 41*]. Myocardial edema can increase cardiac stiffness [*6, 13*], and several investigators have speculated that edema contributes to increased cardiac stiffness after ischemia and reperfusion [*20, 26, 33, 36*]. However, there has been no direct demonstration that reversing myocardial edema during reperfusion after ischemia can reduce cardiac stiffness.

In the present study, increasing colloid osmotic pressure by adding 3% dextran to the coronary perfusate during reperfusion reduced myocardial water content, decreased

* Supported by USPHS Research Grant HL23406.
0022-2828/86/121207+12 $03.00/0

ventricular chamber stiffness, and lowered coronary resistance. These results imply that edema accumulation during reperfusion does contribute to increased cardiac wall stiffness and increased coronary resistance.

Materials and Methods

Experimental preparation

Rabbit hearts were isolated and the coronary arteries perfused by way of the cannulated aortic root, at a constant flow rate of 32 ml/min with oxygenated buffer. A water-filled latex balloon was placed in the left ventricular chamber to keep left ventricular volume constant. The balloon volume was initially adjusted to produce a left ventricular end diastolic pressure (LVEDP) of 10 mmHg; balloon volume was held constant throughout the rest of the experiment. Perfusion pressure and left ventricular pressure were monitored continuously. Heart rate was paced at 180 beats/min. Temperature was maintained at 36.5°C. The perfusion buffer contained (in mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 2.0, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, Na_2 EDTA 0.4, with dextrose 5.5, and sodium lactate 1.0 as substrates. The surgical and perfusion techniques have been described previously in detail [*35*].

Experimental protocol

Two experimental series were undertaken. In the first series dextran (average mol. wt. 82, 200, clinical grade, obtained from Sigma, St. Louis, MO, USA) was added to the perfusate of experimental groups to provide colloid osmotic pressure. This concentration of dextran produced an oncotic pressure of 18 mmHg, measured with a Weil oncometer. Four groups of hearts were studied. Two groups ($n = 6$ each) were perfused after a 30 min equilibration period for another 90 min with no period of ischemia; they received either the control perfusate or 3% dextran during the last 60 min of perfusion. Two groups ($n = 8$ each) were subjected to 30 min of total ischemia (zero coronary flow) after the 30 min equilibration period. Hearts in the control ischemic group were reperfused for 60 min with the standard perfusate. Hearts in the treated ischemic group were reperfused for 5 min with the standard buffer, then perfused with 3% dextran for 50 min, and finally switched back to standard buffer for 5 min. Perfusion with dextran solution was begun after 5 min reperfusion with standard buffer rather than immediately upon reperfusion for two reasons. First, in preliminary studies there was a higher incidence of ventricular fibrillation if hearts were reperfused immediately with 3% dextran solution. Secondly, we wanted to insure that the degree of injury sustained during ischemia was comparable in both groups; perfusion pressure, left ventricular developed pressure, and LVEDP were similar in the two groups at 5 min of reperfusion, indicating that the ischemic insult was comparable before the intervention with dextran. The final return to standard buffer in the treated group was to distinguish possible direct hemodynamic effects of dextran (e.g. due to increased viscosity) from effects due to mobilization of edema.

Ventricular chamber stiffness depends upon the passive elastic properties of the myocardial tissue (inherent stiffness) and also upon coronary arterial pressure and flow (erectile or gardenhose effect) [*6*, *11*, *13*, *21*, *35*, *36*]. To distinguish the contributions of these two factors to chamber stiffness in treated and control hearts, LVEDP was measured at various times, first in the presence of constant coronary flow, then again with perfusion pressure transiently (30 s) reduced to zero. The difference in isovolumic LVEDP measured with and without coronary perfusion was used as an indicator of the vascular erectile contribution to left ventricular chamber stiffness [*36*].

At the end of each experiment, samples of heart tissue (approximately 0.5 g) were taken from the intraventricular septum and the left ventricular free wall. The tissue samples were lightly blotted, minced with scissors, and weighed. The samples were then dried in an oven for 48 h at 40°C and reweighed. The water content was calculated as the difference between wet and dry weight and expressed as ml water/g dry weight. Water content values for septal and free wall samples were similar and, thus, were averaged together for each heart. No attempt was made to measure the intracellular and extracellular spaces by the distribution of conventional markers, such as

inulin, because reperfusion after ischemia can cause gross changes in sarcolemmal permeability [14] which could alter the normal distribution of such markers.

The first series of experiments showed that coronary resistance increased after 30 min of ischemia and that treatment with dextran during reperfusion could decrease coronary resistance. A second series of experiments was undertaken to determine the condition of coronary vasodilator responsiveness after ischemia in this experimental model. Vasodilator responses to adenosine and to hypoxic perfusion were measured by observing the decrease in coronary perfusion pressure at a constant coronary flow rate. Adenosine was infused for 2 min by syringe pump through a side arm of the perfusion cannula, to a final concentration of 10^{-4} M; perfusion pressure was stable after 60 s. After cessation of the adenosine infusion perfusion pressure recovered to basal values within 5 min. At that time perfusion was switched for 2 min to hypoxic buffer (equilibrated with 95% N_2/5% CO_2, $PO_2 < 5$ mmHg). Perfusion pressure decreased within seconds of exposure to hypoxic buffer and was stable after 60 s. Recovery from this transient period of hypoxia was complete within 10 min [2]. In six control hearts responses to hypoxia and adenosine were measured at four times during 75 min of perfusion. In six hearts dilator responses were measured before 15 min of global ischemia and at 15 and 45 min of reperfusion. In another six hearts dilator responses were measured before 30 min of global ischemia and again at 15 and 45 min of reperfusion.

Data analysis

Data are expressed as the mean ± the S.E. of the mean unless otherwise indicated. Mechanical function data from the nonischemic and ischemic-reperfused series were analyzed separately. Differences between control and dextran-treated groups were tested by Student's *t*-test. Differences over time within groups were tested by Student's paired *t*-test. Differences in water content among all groups were tested by one-way analysis of variance; differences between specific group means were tested using a conservative critical "t" value based on Bonferoni's inequality to account for multiple comparisons. The relationships between myocardial edema and changes in coronary resistance and LVEDP were analyzed by linear regression and analysis of covariance.

Results

Myocardial water content

Myocardial water content in the four groups of hearts is shown in Table 1. Differences among the groups were statistically significant ($F = 28.4$, $P < 0.01$). Water content in the reperfused ischemic control group was 18% greater than in the nonischemic control group ($P < 0.001$). Addition of 3% dextran to the perfusate decreased water content in both the nonischemic series (−11%, $P < 0.01$) and in the ischemic-reperfused series (−13%, $P < 0.001$).

Mechanical function in nonischemic hearts

Hemodynamic values for this series of experiments are summarized in Figure 1. Contractile performance of the nonischemic hearts treated with dextran was similar to that of the control hearts. Developed pressure (systolic minus diastolic) decreased slightly during the course of the experiment in both groups. Perfusion with dextran caused a small but statistically significant increase in coronary perfusion pressure. Between 30 and 60 min, perfusion pressure increased by 12 ± 1 mmHg in the dextran-treated group ($P < 0.001$ by paired test), compared to 4 ± 2 mmHg in the control group (n.s. by paired test). There were no statistically significant differences in absolute perfusion pressure between the control and dextran-treated groups. Perfusion with dextran caused a small increase in left ventricular diastolic pressure (LVEDP) compared to the control group. At 60 min, the difference in LVEDP between the groups was 1.9 ± 0.7 mmHg ($P < 0.025$) and at 90 min, 1.8 ± 0.7 mmHg ($P < 0.025$).

TABLE 1. Water content (ml water/g dry weight)

	Nonischemic	Ischemic-reperfused
Control	5.04 ± 0.10	5.99 ± 0.14
Dextran	4.49 ± 0.06	5.22 ± 0.09

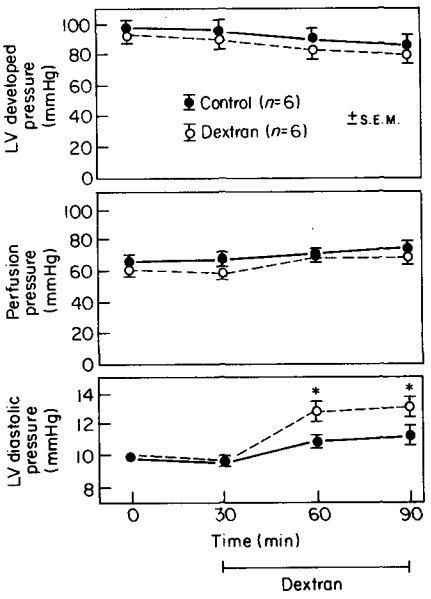

FIGURE 1. Mechanical function in nonischemic hearts. 3% dextran was added to the perfusate of treated hearts at 30 min. The * indicates $P < 0.05$ compared to control group.

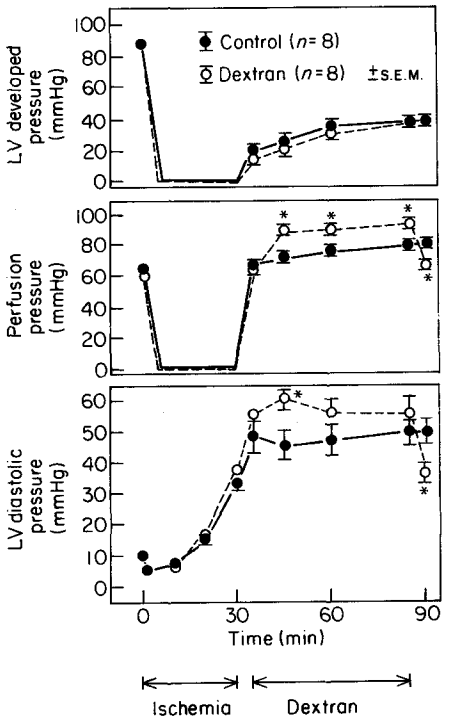

FIGURE 2. Mechanical function in reperfused ischemic hearts. 3% dextran was added to the perfusate of treated hearts between 35 and 85 min. The * indicates $P < 0.05$ compared to control group.

Mechanical function in reperfused ischemic hearts

Hemodynamic values for this series of experiments are summarized in Figure 2. Contractile function was similar in control and dextran-treated hearts. All hearts became asystolic during ischemia; left ventricular developed pressure recovered during 60 min of reperfusion to 46 ± 3% of its initial value in the control group and to 48 ± 4% in the dextran group.

Coronary resistance in the control group increased by 27% during reperfusion, manifested as an increase of coronary perfusion pressure from an initial value of 65 ± 2 mmHg to a value of 82 ± 2 mmHg after 60 min of reperfusion ($P < 0.001$ by paired test). As in the nonischemic series, switching to 3% dextran after 5 min of reperfusion with the standard buffer caused an increase of perfusion pressure. Between 5 and 15 min of reperfusion, coronary perfusion pressure in the dextran group increased by 25 ± 3 mmHg ($P < 0.001$ by paired test) and remained elevated compared to the control group ($P < 0.05$) throughout the dextran perfusion period. When the dextran-treated hearts were switched back to perfusion with the standard buffer, perfusion pressure decreased by 26 ± 2 mmHg to a value significantly lower than that of control reperfused ischemic hearts ($P < 0.05$).

With left ventricular volume held constant by the intraventricular balloon, LVEDP is a measure of ventricular chamber stiffness in this preparation. LVEDP decreased at the onset of ischemia, then gradually increased as the hearts developed ischemic contracture. After 30 min of ischemia, contracture pressure in the two groups was similar. After 5 min of reperfusion, LVEDP increased above the end-ischemia value, by 16 ± 4 mmHg ($P < 0.005$) in the control group and by 18 ± 3 mmHg ($P < 0.001$) in the dextran-treated group. There were no subsequent changes in LVEDP throughout reperfusion in the control group. Switching to 3% dextran after 5 min of reperfusion caused an increase in LVEDP. When the dextran-treated hearts were switched back to the standard buffer, LVEDP decreased by 19 ± 2 mmHg to a

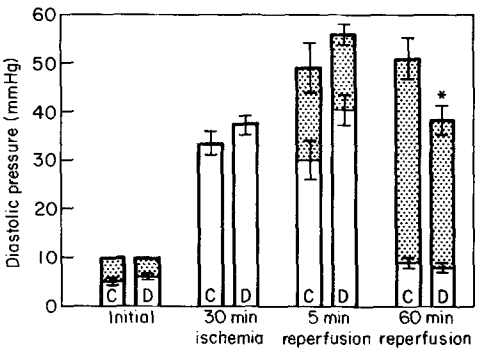

FIGURE 3. Inherent and vascular erectile contributions to left ventricular diastolic pressure in reperfused ischemic hearts. Total bar height indicates left ventricular diastolic pressure measured at a coronary flow rate of 32 ml/min. Open bar represents the inherent component of left ventricular diastolic pressure measured in the absence of coronary perfusion. Shaded portion represents the vascular erectile contribution to diastolic pressure. C = control group; D = dextran group. The * indicates $P < 0.05$ compared to control group. Values are means ±S.E.M. for eight hearts.

value significantly lower than that of the control reperfused ischemic hearts ($P < 0.05$).

Perfusion pressure was transiently (30 s) reduced to zero during the reperfusion period to assess the contribution of coronary perfusion to ventricular chamber stiffness; the results of this procedure are shown in Figure 3. The vascular erectile contribution to LVEDP initially was small and similar in both groups. The increase of LVEDP upon reperfusion was due primarily to the erectile effect of coronary perfusion, since the LVEDP measured when perfusion pressure was reduced to zero after 5 min of reperfusion was similar to that measured at the end of the 30 min ischemic period (i.e., the inherent myocardial stiffness had not increased). After 60 min of reperfusion, the contribution of coronary perfusion to LVEDP increased in both groups. At that time, there was a statistically significant difference in LVEDP between the control and dextran-treated hearts when measured in the presence of coronary perfusion, but there was no difference in LVEDP measured in the absence of coronary perfusion. Thus, the group treated with dextran exhibited a smaller vascular erectile contribution to LVEDP.

The increase in LVEDP during perfusion with dextran in the control and post-ischemic dextran groups and the decrease in LVEDP when perfusion with standard buffer was resumed in the post-ischemic dextran group may have been due to the vascular component of chamber stiffness since perfusion pressure increased during perfusion with the more viscous dextran solution. To test this assumption, the mmHg change in LVEDP per mmHg change in coronary perfusion pressure (ΔEDP/ΔCPP) when the perfusate was changed to or from dextran solution was compared to ΔEDP/ΔCPP observed in the same hearts, just before or after the change of perfusate, when perfusion with the standard buffer was transiently reduced to measure the vascular component of ventricular diastolic pressure. Previous experiments have shown that the relationship between coronary perfusion pressure and LVEDP is linear [*35*]. Figure 4 shows calculated values for ΔEDP/

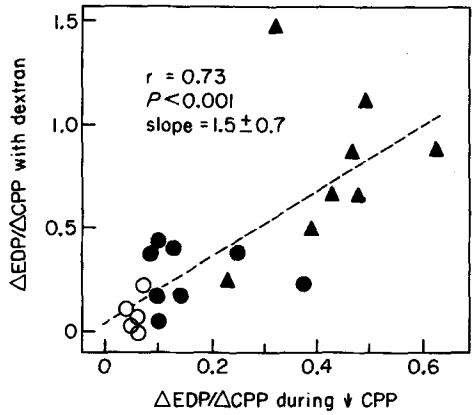

FIGURE 4. Changes in left ventricular diastolic and coronary perfusion pressures with dextran. The mmHg change in LVEDP per mmHg change in coronary perfusion pressure (ΔEDP/ΔCPP) when perfusion was switched between standard buffer and 3% dextran solution is plotted on the vertical axis as a function of ΔEDP/ΔCPP measured in the same hearts when coronary perfusion with standard buffer was transiently decreased to measure the vascular component of LVEDP just before or after the change of perfusate. Each point represents data from one heart at a particular time: (○) switch from standard perfusate to dextran at 30 min in non-ischemic group, (●) switch from standard perfusate to dextran at 35 min in reperfused ischemic group, (▲) switch from dextran back to standard perfusate at 85 min in reperfused ischemic group. Slope of the linear relation does not differ significantly from one. The positive correlation suggests that the changes in coronary perfusion pressure during perfusion with dextran were sufficient to account for the changes in LVEDP.

ΔCPP at these times: (1) when perfusion was switched to dextran solution at 30 min in non-ischemic hearts; (2) when perfusion was switched to dextran solution at 35 min (5 min reperfusion) in post ischemic hearts, and (3) when perfusion was switched back to standard buffer at 85 min. There was a significant linear correlation between ΔEDP/ΔCPP measured when switching to or from dextran and ΔEDP/ΔCPP measured during perfusion with the standard buffer when perfusion pressure was transiently decreased ($r = 0.73$, slope $= 1.5 \pm 0.7$ 95% confidence limits, $P < 0.001$).

Relationships between water content, coronary resistance and LVEDP

To determine whether the decreased coronary resistance and the decreased LVEDP in the dextran group were related to the decrease of myocardial edema, developed pressure, coronary perfusion pressure and LVEDP were plotted as a function of myocardial water content. There was a significant positive correlation in the reperfused ischemic hearts between myocardial water content and coronary perfusion pressure measured 1 h after reperfusion (Fig. 5). There was no statistically

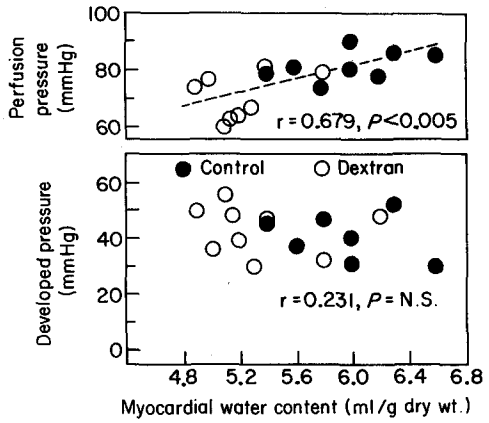

FIGURE 5. Relationships between myocardial water content, perfusion pressure, and developed pressure. Coronary perfusion pressure and left ventricular developed pressure measured 1 h after reperfusion are both plotted as a function of myocardial water content. Each symbol represents values from one heart ($n = 8$ in each group). Dashed line was derived by linear regression; r indicates correlation coefficient.

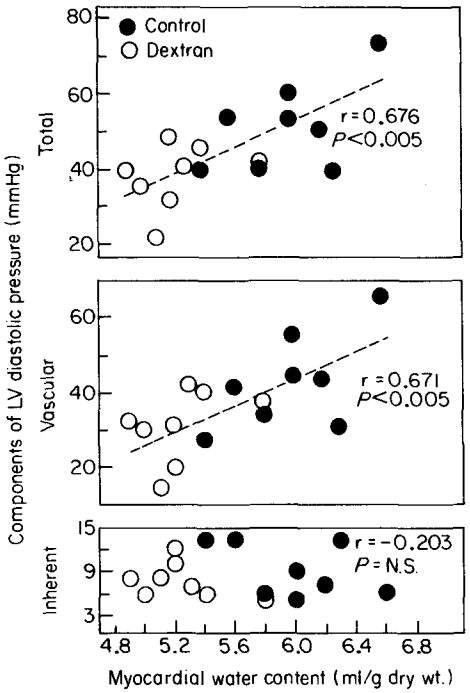

FIGURE 6. Relationships between components of left ventricular diastolic pressure (LVEDP) and myocardial water content. Total LVEDP was measured 1 h after reperfusion with a coronary flow rate of 32 ml/min at a chamber volume which had initially produced LVEDP equal to 10 mmHg during the equilibration period. The inherent component of LVEDP is that measured during transient cessation of coronary perfusion and the vascular component is the difference between LVEDP measured with and without coronary perfusion. Each symbol represents values from one heart ($n = 8$ in each group). Dashed lines were derived by linear regression; r indicates correlation coefficient.

significant correlation between myocardial water content and developed pressure measured 1 h after reperfusion (Fig. 5). There was a positive correlation between myocardial water content and left ventricular diastolic pressure measured 1 h after reperfusion (Fig. 6). There was also a significant correlation between water content and the vascular component of LVEDP but there was not a significant correlation between water content and the inherent component of LVEDP.

Another approach was taken to determine whether the differences in LVEDP and coronary perfusion pressure between untreated and dextran-treated ischemic hearts at 90 min could be ascribed to differences in

myocardial water content. Analysis of covariance was used to see whether any treatment differences remained after adjusting for the linear relationships between LVEDP or coronary perfusion pressure and myocardial water content (Figs 5 and 6). In other words, what would be the treatment differences in LVEDP or coronary perfusion pressure if myocardial water content in the two groups were equal? The absolute difference ($\pm$ standard error of the difference) in LVEDP between treated and untreated hearts was 12.4 ± 5.1 mmHg ($P < 0.05$); the difference in LVEDP after adjusting for the difference in water content was only 1.5 ± 7.7 mmHg ($P > 0.8$). The absolute difference in coronary perfusion pressure between the two groups was 11.0 ± 3.4 mmHg ($P < 0.01$); the adjusted difference was 5.4 ± 5.2 mmHg ($P > 0.2$). Thus, there were not statistically significant differences in LVEDP or coronary perfusion pressure between treated and untreated hearts after adjusting for the difference in water content.

Coronary vasodilator reserve

We considered the possibility that, in addition to mobilizing edema, dextran might have a direct coronary vasodilator effect. To assess the potential for coronary vasodilation during reperfusion we performed a second series of experiments, in which the effect of ischemia on coronary vasodilator reserve in this experimental model was examined by measuring dilator responses (decrease in perfusion pressure with constant coronary flow rate) during 2 min of perfusion with hypoxic buffer or 10^{-4} M adenosine. Dilator responses in control hearts and hearts subjected to 15 or 30 min of ischemia with 45 min of reperfusion are shown in Figure 7. The initial dilator responses to hypoxia or adenosine were similar in the three groups. The initial dilator response to hypoxia was slightly but consistently greater than the response to adenosine ($P < 0.02$ in each group for adenosine v. hypoxia responses by paired t-test). In control hearts, coronary perfusion pressure increased gradually during 75 min of perfusion but the dilator responses to hypoxia and to adenosine remained stable. During reperfusion, after 15 min of ischemia, vasodilator responses to both hypoxia and adenosine were retained. During reperfusion after 30 min of ischemia, vasodilator responses were markedly reduced; at 45 min of reperfusion, the response to hypoxia was only 28% of the initial response ($P < 0.025$ for 45 min reperfusion vs. initial value by paired t-test) and there was no response to adenosine ($P < 0.02$ for 45 min reperfusion v. initial value by paired t-test). These results suggest that dextran was unlikely to have decreased coronary resistance by a direct vasodilating effect, since coronary vasomotion was markedly impaired following 30 min of ischemia and reperfusion. Thus, these results are consistant with mobilization of edema as the cause of the decrease in coronary resistance in the dextran-treated reperfused ischemic group.

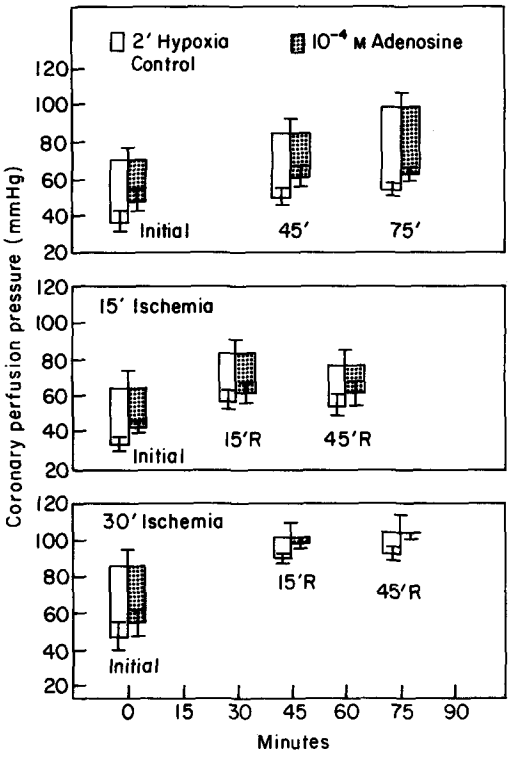

FIGURE 7. Vasodilator responses in control and reperfused ischemic hearts. Top of each pair of bars indicates baseline coronary perfusion pressure. Length of bar indicates decrease in perfusion pressure (coronary flow constant) during perfusion with hypoxic buffer or adenosine. R = reperfusion. Values are presented as mean $\pm$S.E.M. for six hearts in each group.

Discussion

Timely reperfusion of ischemic myocardium can salvage viable myocytes, but reperfusion also accelerates the expression of ischemic injury in non-viable cells and may impose additional injury on jeopardized cells. Reperfusion of ischemic myocardium is associated with a variety of myocardial changes [*14*, *15*, *17*], including marked myocardial edema [*17*]. Several investigators have speculated that myocardial edema may contribute to increased coronary arterial resistance and decreased cardiac distensibility, which occur during reperfusion [*7*, *18*, *20*, *23*, *26*]. Edema unrelated to ischemic/reperfusion injury is associated with increased ventricular chamber stiffness [*6*, *13*]. Pharmacological interventions, such as mannitol and steroids, which may act in part by decreasing edema [*8*, *41*], have been reported to decrease coronary resistance and cardiac stiffness in the setting of experimental ischemia [*8*, *9*, *33*, *40*]. But results with these reagents are inconsistent [*38*, *40*] and both may be acting through direct vasodilator effects [*38*, *40*], or other actions including membrane stabilization [*9*], positive inotropic activity [*32*, *40*], and free radical scavenging [*15*]. Moreover, these agents have usually been administered during ischemia and may be moderating ischemic rather than reperfusion injury. Increasing osmolarity with mannitol or increasing colloid osmotic pressure with albumin decreased the myocardial edema and attenuated the increased cardiac stiffness which occurred during brief cardioplegic arrest with crystalloid solutions [*10*]. No experiments have been reported demonstrating a decrease in cardiac stiffness by decreasing myocardial edema during reperfusion after ischemia. The present study was designed to determine the effects of increasing colloid osmotic pressure with dextran during reperfusion.

Addition of dextran to the perfusate decreased water content by a similar amount in nonischemic and reperfused ischemic hearts. Nonischemic hearts become edematous in such a preparation [*1*, *22*], due to the lack of colloid osmotic pressure in the perfusate and due to the arteriolar vasodilation which attends the low oxygen content of the perfusate. Nevertheless, there was a further significant increase of water content which occurred as a result of ischemic and reperfusion injury. The percentage increase of water content due to ischemia and reperfusion in the present study (18%) was in the range observed after ischemic arrest of blood-perfused hearts [*8*, *9*, *12*, *20*, *34*, *37*].

Addition of dextran to the perfusate increased perfusion pressure and LVEDP in both normal and reperfused ischemic hearts. The increases were greater in the reperfused ischemic hearts. The increase in perfusion pressure was probably due to the increased viscosity of the dextran solution [*3*]. The increase of LVEDP during perfusion with dextran may have followed from the effect on coronary perfusion pressure. In addition to the inherent elastic properties of the heart wall, there is a contribution of coronary perfusion to LVEDP [*6*, *11*, *13*, *21*, *34*, *35*]. This vascular "erectile" or "garden-hose" contribution to LVEDP is markedly increased after ischemia and reperfusion [*11*, *34*, *35*]. The $\Delta EDP/\Delta CPP$ when switching to or from perfusion with dextran solution correlated with $\Delta EDP/\Delta CPP$ when perfusion pressure was transiently reduced to measure the magnitude of the vascular contribution to LVEDP. Switching back from 3% dextran to the standard perfusate after 60 min of reperfusion removed the immediate physical effects of dextran, revealing decreased coronary resistance and decreased LVEDP compared to untreated reperfused ischemic hearts. Linear regression analysis demonstrated a significant correlation between myocardial water content and the increases in both perfusion pressure and LVEDP. Altering myocardial water content selectively affected the vascular erectile contribution to LVEDP with no effect on inherent chamber stiffness (Figs 3 and 5). The decreases in coronary resistance and LVEDP in the dextran-treated group appear to be specific consequences of reducing myocardial water content, rather than some general effect of dextran on myocardial recovery, because there was no correlation between myocardial water content and the recovery of developed pressure. Furthermore, analysis of covariance showed that no significant differences in LVEDP or coronary perfusion pressure remained between treated and untreated isch-

emic groups after adjustment for the difference in myocardial water content.

The second series of experiments showed that coronary vasodilator capacity was severely compromised after 30 min of global ischemia in this preparation; 45 min after reperfusion there was only a small dilator response to hypoxia and none to 10^{-4} M adenosine. The loss of coronary vasodilator responsiveness was related to the duration of ischemia since normal dilator responses were retained after 15 min of ischemia. This is consistent with previous results of others. Elzinga *et al.* [*7*] reported normal coronary reactive hyperemic responses after 20 min coronary occlusion in dogs, but markedly attenuated dilator responses after 60 or 120 min occlusion. Willerson *et al.* [*39*] observed normal coronary dilator responses to organic nitrates and adenosine after 40 min of coronary occlusion and 20 min reperfusion in a canine model despite the presence of interstitial and intracellular myocardial edema. The same laboratory [*41*] reported a reduction in reflow to the ischemic bed after 120 min of coronary occlusion; mannitol, but not organic nitrates, was effective in lowering the increased resistance of the reperfused vascular bed. Loss of coronary vasomotor function might be a slower process in the canine coronary occlusion model than in our global ischemia model due to significant collateral coronary flow in the former situation. Because vascular smooth muscle cells are quite permeable and behave like an osmometer, increasing oncotic pressure with dextran could elicit active vasodilation by decreasing intracellular water, thus increasing intracellular K^+ concentration of vascular smooth muscle cells [*30*]. However, active dilation would seem to be an unlikely explanation for the decrease in coronary resistance after dextran in the present study since active vasodilation could not be elicited by adenosine. It is more likely that the decrease in coronary resistance after dextran treatment was secondary to decreased vascular compression accompanying the decrease in ventricular diastolic pressure. The strength of the correlation between water content and coronary perfusion pressure (Fig. 5) suggests that about half of the variation in coronary resistance was connected with variation in water content ($r^2 = 0.46$). Other factors which might contribute to increased coronary resistance after ischemia include foci of myocardial contraction band necrosis and direct vascular endothelial injury [*16*].

Our observations may be applicable to clinical settings of ischemia and reperfusion. The change in LVEDP for a particular increment of myocardial water content which we observed in these buffer-perfused hearts (16 mmHg change in LVEDP for a 1 ml/g dry wt change in water content) is similar to the relationship observed [*6, 10, 20*] in blood-perfused hearts (8 to 16 mmHg/ml H_2O/g dry wt) with edema induced by a variety of methods. As an illustration, our data are compared in Figure 8 to values calculated from the data of Cross *et al.* [*6*], who observed a linear increase of isovolumic LVEDP as heart weight increased due to edema accumulation in blood-perfused dog hearts. Experimental ischemic cardiac arrest and reperfusion can increase myocardial water content by 0.5 to 0.8 ml/g dry wt [*8, 9, 12, 20, 34, 37*]. Hypothermia during ischemic arrest does not prevent the accumulation of edema [*9, 20, 34*], and procedures such as cardiopulmonary bypass, hemodilution, and the use of crystalloid cardioplegic solutions can also induce significant myocardial edema [*10, 19, 27, 28*]. Thus myocardial edema, observed in patients who had undergone ischemic cardioplegic arrest [*28*], may contribute to the increased ventricular chamber stiffness observed in such patients [*5, 29, 31*]. Our results indicate that decreasing myocardial water content during reperfusion can decrease elevated ventricular chamber stiffness. Although increasing colloid osmotic pressure above normal to decrease myocardial water content may not be practicable, other measures could be taken during cardiac surgery to avoid excessive myocardial edema. Such measures might include: using colloid rather than crystalloid solutions for cardioplegia [*10, 28*] and hemodilution [*19, 27*], restoring significantly decreased colloid osmotic pressures during surgery to normal [*27*], and avoiding excessive perfusion pressures during reperfusion [*24*].

In summary, these results indicate that edema plays a significant role in maintaining increased ventricular chamber stiffness and increased coronary resistance during per-

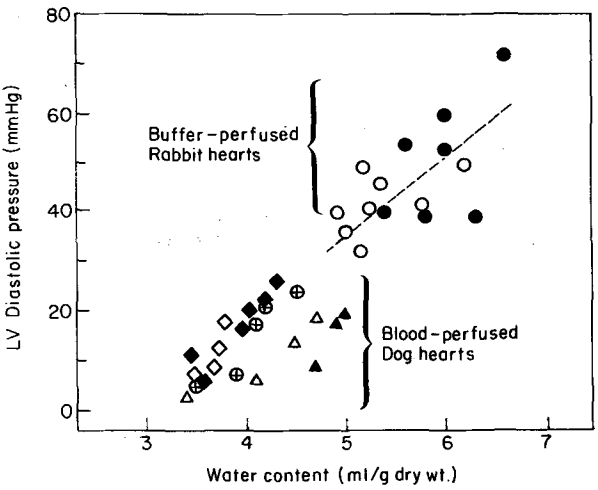

FIGURE 8. Relationships between left ventricular diastolic pressure (LVEDP) and myocardial water content in buffer-perfused and blood-perfused hearts. Circles represent data from the present study measured 1 h after reperfusion in untreated (●) and dextran-treated (○) hearts; each symbol represents values for one heart. Data from blood-perfused hearts were calculated from data of Cross *et al.* [5]. Data from five hearts are shown with different symbols (◇, ◆, △, ▲, ⊕) indicating values for different individual hearts; several values of LVEDP were measured in each heart during progressive increases of myocardial water content. The increase in LVEDP for a given increase in water content is similar in blood-perfused and buffer-perfused hearts.

fusion after myocardial ischemia. Decreasing myocardial water content decreased chamber stiffness and coronary resistance. This suggests that care should be taken to avoid excessive myocardial edema during surgical ischemic arrest and reperfusion.

References

1 Apstein, C. S., Mueller, M., Hood, W. B. Jr Ventricular contracture and compliance changes with global ischemia and reperfusion and their effect on coronary resistance in the rat. Circ Res **41**, 206–217 (1977).

2 Apstein, C. S., Vogel, W. M. Coronary vasodilator effect of ibuprofen. J Pharmacol Exp Ther **220**, 167–171 (1982).

3 Arnold, G., Kosche, F., Miessner, E., Neitzert, A., Lochner, W. The importance of the perfusion pressure in the coronary arteries for the contractility and the oxygen consumption of the heart. Pflugers Arch **299**, 339–356 (1968).

4 Bersohn, M. M., Shine, K. I., Sterman, W. D. Effect of increased magnesium on recovery from ischemia in rat and rabbit hearts. Am J Physiol **242** (Heart Circ Physiol **11**), H89–H93 (1982).

5 Chitwood, W. R., Hill, R. C., Sink, J. D., Wechsler, A. S. Diastolic ventricular properties in patients during coronary revascularization. J Thorac Cardiovasc Surg **85**, 595–605 (1983).

6 Cross, C. E., Rieben, P. A., Salisbury, P. F. Influence of coronary perfusion and myocardial edema on pressure-volume diagram of left ventricle. Am J Physiol **201**, 102–108 (1961).

7 Elzinga, W. E., Skinner, D. B., Dutka, M. F., Gott, V. L. Coronary blood flow and myocardial reactive hyperemia in the ischemic dog heart. Surgery **76**, 482–489 (1974).

8 Feola, M., Rovetto, M., Soriano, R., Cho, S. Y., Wiener, L. Glucocorticoid protection of the myocardial cell membrane and the reduction of edema in experimental acute myocardial ischemia. J Thorac Cardiovasc Surg **72**, 631–643 (1976).

9 Fey, K. H., Follette, D., Livesay, J., Nelson, R., Bugyi, H., DeLand, E., Buckberg, G. D. Effects of membrane stabilization on the safety of hypothermic arrest after aortic cross-clamping. Circulation **56** [Suppl II], II-143–II-147 (1977).

10 Foglia, R. P., Steed, D. L., Follette, D. M., DeLand, E., Buckberg, G. D. Iatrogenic myocardial edema with potassium cardiplegia. J Thorac Cardiovasc Surg **78**, 217–222 (1979).

11 Gaasch, W. H., Bing, O. H. L., Franklin, A., Rhodes, D., Bernard, S. A., Weintraub, R. N. The influence of acute alterations in coronary blood flow on left ventricular diastolic compliance and wall thickness. Eur J Cardiol **7** [Suppl], 147–161 (1978).

12 GAASCH, W. H., BING, O. H. L., PINE, M. B., FRANKLIN, A., CLEMENT, J., RHODES, D., PHEAR, W. B., WEINTRAUB, R. N. Myocardial contracture during prolonged ischemic arrest and reperfusion. Am J Physiol **235** (Heart Circ Physiol **4**), H619–H627 (1978).
13 GREUNER-SIGUSCH, P., GREUNER, G., MORGENSTERN, C. Der Einfluss des Druckes in den Coronararterien auf die Druck-Volumen-Beziehung des linken Ventrikels. Pflügers Arch **338**, 233–246 (1973).
14 HEARSE, D. J. Reperfusion of the ischemic myocardium. J Mol Cell Cardiol **9**, 605–613 (1977).
15 HESS, M. L., MANSON, N. H. Molecular oxygen: Friend and foe; The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J Mol Cell Cardiol **16**, 969–985 (1984).
16 KLONER, R. A., GANOTE, C. E., JENNINGS, R. B. The 'no-reflow' phenomenon after temporary coronary occlusion in the dog. J Clin Invest **54**, 1496–1508 (1974).
17 KLONER, R. A., GANOTE, C. E., WHALEN, D. A., JENNINGS, R. B. Effect of a transient period of ischemia on myocardial cells. II. Fine structure during the first few minutes of reflow. Am J Pathol **74**, 399–422 (1974).
18 KRUG, A., DUMESNIL DEROCHEMONT, W., KORB, G. Blood supply of the myocardium after temporary coronary occlusion. Circ Res **19**, 57–62 (1966).
19 LAKS, H., STANDEVEN, J., BLAIRE, O., HAHN, J., JELLINEK, M., WILLMAN, V. L. The effects of cardiopulmonary bypass with crystalloid and colloid hemodilution on myocardial extravascular water. J Thorac Cardiovasc Surg **73**, 129–138 (1977).
20 NELSON, R. L., GOLDSTEIN, S. M., MCCONNELL, D. H., MALONEY, J. V., BUCKBERG, G. D. Studies of the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass: V. Profound topical hypothermia during ischemia in arrested hearts. J Thorac Cardiovasc Surg **73**, 201–207 (1977).
21 OLSEN, O., ATTARIAN, D. E., JONES, R. N., HILL, R. C., SINK, J. D., LEE, K. L., WECHSLER, A. S. The coronary pressure-flow determinants of left ventricular compliance in dogs. Circ Res **49**, 856–865 (1981).
22 OPIE, L. H., SHIPP, J. C., EVANS, J. R., LEBOEUF, B. Metabolism of glucose-U-C^{14} in perfused rat heart. Am J Physiol **203**, 839–843 (1962).
23 PARKER, P. E., BASHOUR, F. A., DOWNEY, H. F., KECHEJIAN, S. J., WILLIAMS, A. G. Coronary hemodynamics during reperfusion following acute coronary ligation in dogs. Am Heart J **90**, 593–599 (1975).
24 PENG, C. F., KANE, J. J., JONES, E. M., MURPHY, M. L., STRAUB, K. D., DOHERTY, J. E. The adverse effect of systemic hypertension following myocardial reperfusion. J Surg Res **34**, 59–67 (1983).
25 PIRZADA, F. A., WEINER, J. M., HOOD, W. B. Jr Experimental myocardial infarction. 14. Accelerated myocardial stiffening related to coronary reperfusion following ischemia. Chest **74**, 190–195 (1978).
26 SCHAFF, H. V., GOTT, V. L., GOLDMAN, R. A., FREDERICKSEN, J. W., FLAHERTY, J. T. Mechanism of elevated left ventricular end-diastolic pressure after ischemic arrest and reperfusion. Am J Physiol **240** (Heart Circ Physiol **9**), H300–H307 (1981).
27 SHIPPY, C. R., SHOEMAKER, W. C. Hemodynamic and colloid osmotic pressure alterations in the surgical patient. Crit Care Med **11**, 191–195 (1983).
28 SINGH, A. K., FARRUGIA, R., TEPLITZ, C., KARLSON, K. E. Electrolyte versus blood cardiplegia: Randomized clinical and myocardial ultrastructural study. Ann Thorac Surg **33**, 218–227 (1982).
29 SLACK, J. D., ZEOK, J. V., COLE, J. S., HANLEY, H. G., CORNISH, A. L., MCKEAN, H. E. Influence of potassium cardiplegia versus ischemic arrest on regional left ventricular diastolic compliance in humans. Ann Thorac Surg **31**, 214–223 (1981).
30 SPARKS, H. V. Effect of local metabolic factors on vascular smooth muscle. In *Handbook of Physiology, Section 2: The Cardiovascular System, Volume II Vascular Smooth Muscle.* D. F. Bohr, A. P. Somlyo, H. V. Sparks Jr (Eds), pp. 475–513, Bethesda, MD: American Physiological Society (1980).
31 SPOTNITZ, H. M., BREGMAN, D., BOWMAN, F. O. Jr, EDIE, R. N., REEMTSMA, K., KING, D. L., HOFFMAN, B. F., MALM, J. R. Effects of open heart surgery on end-diastolic pressure-diameter relations of the human left ventricle. Circulation **59**, 662–671 (1979).
32 TECKLENBERG, P. L., MULLIN, E. M., STINSON, E. B., MORROW, A. G. The effects of massive doses of methylprednisolone on myocardial contractility and peripheral vascular resistance. Am Heart J **85**, 216–226 (1973).
33 TOYAMA, M., REIS, R. L. Effects of myocardial ischemia on ventricular compliance, protective role of hydrocortisone. J. Thorac Cardiovasc Surg **70**, 458–465 (1975).
34 UTLEY, J. R., MICHALSKY, G. B., BRYANT, L. R., MOBIN-UDDIN, K., MCKEAN, H. E. Determinants of myocardial water content during cardiopulmonary bypass. J Thorac Cardiovasc Surg **68**, 8–16 (1974).
35 VOGEL, W. M., APSTEIN, C. S., BRIGGS, L. L., GAASCH, W. H., AHN, J. Acute alterations in left ventricular diastolic chamber stiffness: Role of the 'erectile' effect of coronary arterial pressure and flow in normal and damaged hearts. Circ Res **51**, 465–478 (1982).
36 VOGEL, W. M., BRIGGS, L. L., APSTEIN, C. S. Separation of inherent diastolic myocardial fiber tension and coronary vascular erectile contributions to wall stiffness of rabbit hearts damaged by ischemia, hypoxia, calcium paradox, and reperfusion. J Mol Cell Cardiol **17**, 57–70 (1985).
37 VOGEL, W. M., LUCCHESI, B. R. An isolated blood-perfused feline heart preparation for evaluating pharmocological interventions during myocardial ischemia. *J Pharm Meth* **4**, 291–303 (1980).
38 VOGEL, W. M., LUM, D., LUCCHESI, B. R. Methylpredinisolone sodium succinate treatment in global ischemia of the cat isolated heart. J Cardiovasc Pharmacol **1**, 53–68 (1979).
39 WILLERSON, J. T., SCALES, F., MUKHERJEE, A., PLATT, M., TEMPLETON, G. H., FINK, G. S., BUJA, L. M. Abnormal myocardial fluid retention as an early manifestation of ischemic injury. Am J Pathol **87**, 159–188 (1977).

40 WILLERSON, J. T., WATSON, J. T., HUTTON, I., FIXLER, D. E., CURRY, G. C., TEMPLETON, G. H. The influence of hypertonic mannitol on regional myocardial blood flow during acute and chronic myocardial ischemia in anesthetized and awake intact dogs. J Clin Invest **55**, 892–902 (1975).

41 WILLERSON, J. T., WATSON, J. T., HUTTON, I., TEMPLETON, G. H., FIXLER, D. E. Reduced myocardial reflow and increased coronary vascular resistance following prolonged myocardial ischemia in the dog. Circ Res **36**, 771–781 (1975).