Increased Diastolic Chamber Stiffness During Demand Ischemia

Response to Quick Length Change Differentiates Rigor-Activated From Calcium-Activated Tension

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Background—Increased diastolic chamber stiffness (↑DCS) during angina (demand ischemia) has been postulated to be generated by increased diastolic myocyte calcium concentration.

Methods and Results—We reproduced demand ischemia in isolated isovolumically contracting red-cell–perfused rabbit hearts by imposing pacing tachycardia during global low coronary blood flow (32% of baseline). This increased lactate production without increasing oxygen consumption and resulted in ↑DCS (isovolumic left ventricular end-diastolic pressure [LVEDP] increased 10 mm Hg, P<0.001, n=38). To determine the mechanism of ↑DCS, we assessed responses to a quick-stretch-release maneuver (QSR), in which the intraventricular balloon was rapidly inflated and deflated to achieve a 3% circumferential muscle fiber length change. QSR was first validated as an effective method of discriminating between calcium-driven and rigor-mediated ↑DCS. QSR imposed during demand ischemia when DCS had increased (LVEDP pretachycardia versus posttachycardia, 15±6 versus 27±2 mm Hg, P<0.001, n=6) reduced DCS to pretachycardia values (LVEDP post-QSR, 15±1 mm Hg, P<0.001), ie, elicited a response characteristic of rigor, without any component of calcium-generated tension.

Conclusions—A rigor force, possibly resulting from high-energy phosphate depletion and/or an increase in ADP, appears to be the primary mechanism underlying increased DCS in this model of global LV demand ischemia. (Circulation. 2000;101:2185-2192.)

Key Words: diastole ■ angina ■ rigor
Demand Ischemia

Demand ischemia was reproduced by imposition of combined ischemia and tachycardia to increase metabolic demand (n=38).

To impose ischemia, coronary blood flow was reduced to achieve a coronary perfusion pressure of 20 mm Hg, and flow was then held constant at this level. This degree of coronary perfusion pressure is comparable to the perfusion pressure distal to severe coronary stenoses in patients with angina.18

5 imposed (n=5) and freeze-clamped when LVEDP had increased 10 mm Hg while pacing was continued. Then, when isovolumic LVEDP had increased 10 mm Hg, QSR was performed, and then...
LVEDP occurred in all hearts (mean, 15.3 mm Hg) followed by a more gradual increase in LVEDP. A rise in isovolumic LVEDP (incomplete relaxation), followed by a more gradual increase in LVEDP. A rise in LVEDP occurred in all hearts (mean, 15.3 ± 0.9 mm Hg) after LVEDP had increased 10 mm Hg. tachycardia was terminated (pacing rate returned to 3 Hz), but coronary flow continued at its ischemic level. In this phase, LVEDP remained elevated by 7 ± 1 mm Hg compared with pretachycardia levels (P<0.001), representing a stable increase in diastolic chamber stiffness resulting from increased metabolic demand during low-flow perfusion.

**Reversibility**

In hearts undergoing reperfusion (n=7), LVEDP returned to baseline (22±2 mm Hg) and LVSP recovered (88±4 mm Hg) within 5 minutes, indicating the rapidly reversible nature of the ischemic increase in diastolic chamber stiffness.

Hearts subjected to tachycardia without ischemia or to ischemia without tachycardia demonstrated no significant increase in LVEDP (Figure 2a). Thus, neither tachycardia alone nor low-flow ischemia alone was sufficient, but the combination, ie, demand ischemia, resulted in increased diastolic chamber stiffness.

**Metabolic Characteristics**

With the onset of ischemia (demand ischemia and ischemia without tachycardia groups), hearts switched from net myocardial lactate consumption to net lactate production (Figure 2b and 2c). In ischemia without tachycardia, lactate production then remained constant. In contrast, in demand ischemia, tachycardia further increased lactate production (demand ischemia, 0.45±0.03 versus ischemia without tachycardia, 0.12±0.03 [μmol/L] · mL⁻¹ · min⁻¹ · g LV wet wt⁻¹, P<0.005). Lactate production was also greater in demand ischemia than in hearts subjected to tachycardia without ischemia.

Oxygen consumption decreased in both demand ischemia and ischemia without tachycardia. Imposition of tachycardia in demand ischemia did not alter oxygen consumption. In tachycardia without ischemia oxygen, conversely, consumption increased during tachycardia. Hence, hearts in demand ischemia in which coronary flow was restricted were unable to increase oxygen consumption when energy demand was increased by tachycardia, in contrast to the group in which coronary flow was allowed to increase commensurately with increased metabolic demand.

**ATP Content**

We have previously reported a baseline ATP content of 18.01±2.00 μmol/L ATP/g LV dry wt in this experimental preparation. Hearts subjected to demand ischemia (isovolumic LVEDP pretachycardia versus posttachycardia [15±2 minutes], 17±1 versus 26±1 mm Hg, P<0.001) had an ATP content of 9.12±1.76 (μmol/L)/g LV dry wt, ie, a 50% reduction at the point at which LVEDP had increased 10 mm Hg. In ischemia without tachycardia (ischemia duration, 14±3 minutes), LVEDP remained unaltered, and ATP content was 10.78±0.94 (μmol/L)/g LV dry wt (P=NS versus demand ischemia). Thus, these 2 groups did not differ in end-ischemic [ATP; despite the imposition of tachycardia and the development of increased diastolic chamber stiffness in the demand ischemia group.

**Quick-Stretch-Release**

Groups of hearts in which QSR was performed had similar baseline hemodynamic characteristics before interventions were performed (Figures 3 through 7). QSR at baseline...
(normoxia, n=7) did not affect function (pre-QSR versus post-QSR. LVEDP, 20±1 versus 19±1 mm Hg, P=NS; LVSP, 122±3 versus 121±4 mm Hg, P=NS) (Figure 3).

With zero-flow ischemia (n=6), hearts rapidly became asystolic and LVEDP initially decreased (16±1 mm Hg) (Figure 4; Reference 21). During sustained ischemia (18±4 minutes), LVEDP progressively increased, resulting in ischemic contracture ("classic rigor") 

QSR at this point instantly lysed this rigor tension (LVEDP pre-QSR versus post-QSR, 27±1 versus 17±1 mm Hg, P<0.001), ie, LV diastolic pressure decreased to precontracture values with no tension recovery.

When increased diastolic chamber stiffness occurring from increased cytosolic diastolic calcium was created by intracoronary infusion of caffeine and calcium chloride (n=6, Figure 5), LVEDP increased from a baseline of 16±1 to 28±1 mm Hg (P<0.001). QSR imposed at this point had no effect on increased diastolic tension or systolic function (pre-QSR versus post-QSR: LVEDP, 27±1 versus 26±1 mm Hg, P=NS; LVSP, 111±5 versus 109±5 mm Hg).

Figure 3. QSR at baseline. Coronary artery perfusion pressure (CPP) was 85 mm Hg (normoxia). LVEDP before and immediately after QSR was 20 mm Hg, ie, unaffected. LVSP and differentiated pressure (dP/dt) increased slightly for a few cycles after QSR before returning to baseline.

Figure 4. QSR during classic rigor. Zero-flow ischemia (coronary perfusion pressure [CPP] of zero) resulted in contractile failure (LVSP of zero). An initial reduction in LVEDP from 20 to 17 mm Hg was followed by a progressive increase, ie, increased diastolic chamber stiffness due to rigor contracture. When LVEDP had increased from 17 to 27 mm Hg, QSR caused immediate reduction to 17 mm Hg, ie, LVEDP returned to precontracture values. Note different paper speeds. During development of contracture, speed is 0.05 mm/s.
LVEDP, however, returned to baseline values on termination of infusion. Hence, the different responses of increased LVEDP to QSR between rigor bonds in classic rigor compared with a calcium-driven mechanism validated QSR as a method of discriminating rigor- versus calcium-mediated increases in diastolic chamber stiffness.

In demand ischemia, tachycardia was terminated after LVEDP had increased from 15±1 to 27±2 mm Hg (P<0.001, n=6). QSR subsequent to tachycardia immediately lysed increased diastolic tension (LVEDP pre-QSR versus post-QSR, 27±2 versus 15±1 mm Hg, P<0.001), ie, chamber stiffness returned to baseline (Figure 6). The decrement of LVEDP produced by QSR was identical in magnitude to the upward shift of isovolumic LVEDP sustained during pacing tachycardia. Hence, QSR during demand ischemia elicited a response similar to that with rigor contraction associated with zero-flow ischemia but unlike that with calcium-activated increased diastolic tension (Figure 7).

**Discussion**

The acute and reversible decrease in diastolic distensibility during angina due to sustained actin-myosin interaction during diastole may be related to myocardial ATP depletion (with a concomitant increase in ADP) resulting in rigor, to diastolic persistence of an increased intracellular calcium concentration, or to a combination of the 2. In the present...
study using a model simulating some of the features of

demand ischemia during angina, responses to quick length
changes support a rigor mechanism, without involvement of a
calcium-driven tension.

In this model, we reproduced reversible diastolic dysfunc-
tion with demand ischemia: isovolumic LVEDP increased
with combined low-flow ischemia and tachycardia but not
with either ischemia or tachycardia alone and was character-
ized by increased lactate production with an inability to
increase oxygen consumption (Figures 1 and 2). Here, the
globally ischemic LV served to model the regionally ische-
mic region in a patient with angina or a large-animal model
with single coronary artery stenosis. This, by imposing
homogeneous conditions throughout the LV, facilitated de-
termination of mechanisms underlying diastolic dysfunction.
However, reduction of coronary artery perfusion pressure
before tachycardia, with a marked global reduction in coro-

nary artery flow, resulted in profound contractile dysfunction.
Systolic dysfunction of this degree does not usually accom-
pany regional ischemia or angina unless there is also severe
global ischemia, eg, left main or 3-vessel disease, severe
aortic stenosis, or systemic hypotension and tachycardia.
Hence, our model does not simulate all the hemodynamic
features observed during regional demand ischemia in hu-
mans or large-animal models (in which resting coronary flow
in a stenotic segment and contractile function may remain
unchanged), and this limitation prevents direct extrapolation
of our results to clinical angina.

Quick-Stretch-Release

The QSR maneuver, in which a sudden increment (1% to
10%) in length is followed by a rapid return to baseline
length, has been used to distinguish between calcium-acti-
vated and rigor tension in unstimulated skeletal16 and papil-
lary18 muscle. After QSR, rigor bonds are characterized by
tension lysis and by failure of immediate tension redevelop-
ment, so that poststretch tension remains markedly reduced
relative to the prestretch level. In contrast, QSR imposed on

muscle with continuous calcium-activated cross-bridge cy-
cling is followed by incomplete lysis and by a rapid redevel-
opment of tension to its prestretch value.

Here, QSR was similarly applied in the actively contracting
isolated heart without deleterious effects (Figure 3). During
tonic contracture due to classic ischemic rigor, QSR caused
immediate, complete, and sustained lysis of diastolic tension
(Figure 4), but when an increase in diastolic chamber stiffness
was driven by diastolic persistence of calcium, QSR failed to
alter diastolic tension (Figure 5).

Thus, we could examine effects of QSR under specific
conditions in which diastolic dysfunction occurred in con-
tracting hearts. We hypothesized that in demand ischemia, if
increased LVEDP were produced by persistent cross-bridge
cycling, equivalent to a state of sustained partial systole due
to diastolic persistence of increased calcium, then QSR would
cause no significant lysis of diastolic tension. Conversely, if
rigor force were responsible, QSR should effectively lyse this
tension. If a combination of these mechanisms were opera-
tive, then an intermediate response would be predicted. In our
model, QSR produced complete lysis of increased diastolic
tension resulting from demand ischemia, a behavior typical of
rigor without any component of a calcium-driven tension
(Figures 6 and 7).

Subcellular Mechanisms of Increased
Diastolic Tension

The subcellular mechanisms underlying increased chamber
stiffness in demand ischemia have received relatively little
study. One report proposed a mechanism of increased dia-

stolic myocyte calcium concentration based on the observa-
tion that exposure to caffeine during the last 30 seconds of
pacing tachycardia exacerbated the degree of increased dia-
stolic chamber stiffness sustained during demand ischemia.6
However, this conclusion is confounded because caffeine
itself may have contributed importantly to cytosolic calcium
overload. For example, increased diastolic chamber stiffness
can occur in normoxic hearts on exposure to caffeine: in our
experimental model (Figure 5), caffeine increased LVEDP,
which was further exaggerated by superimposed calcium
loading. Thus, the observation that caffeine augmented an
increase in ischemic diastolic stiffness does not prove that the
initial ischemia-induced increase in stiffness itself was
calcium-driven.

In contrast, many previous studies have investigated the
mechanism of ischemic contracture in a variety of models, eg,
hearts in situ or subjected to hypoxia or zero-flow ischemia,
or isolated muscle strips or myocytes subjected to metabolic
inhibition. Under these conditions, an increase in diastolic
calcium level has been widely reported,7,8 consistent with a
calcium-driven mechanism for the contracture. This remains
an appealing explanation, although no cause-and-effect rela-
tion has been definitively established. Others report no
correlation between increased myocyte calcium and increased
diastolic tension9,22 and favor an alternative mechanism of
rigor.9–11,23 These models, however, comprise a heteroge-

neous group of ischemic states, and their results may not be
readily extrapolated to the demand ischemia of clinical
angina.
Our result of diastolic tension lysis by QSR during demand ischemia supports a rigor mechanism secondary to ATP depletion as the basis of increased diastolic chamber stiffness. However, we could not demonstrate a lower average tissue [ATP] in hearts subjected to demand ischemia (in which an increase in diastolic chamber stiffness occurred) compared with hearts subjected to similar ischemia but without tachycardia, in which no increase in diastolic tension occurred. In both groups, [ATP] decreased by only 50%. Thus, we could not correlate the increase in ischemic diastolic tension with total tissue ATP depletion.

However, these ATP measurements do not rule out rigor tension as the mechanism for the increase in ischemic diastolic chamber stiffness. Rigor tension may be generated in the presence of only modest reductions in [ATP] when [ADP] increases\textsuperscript{12–14} and may be correlated with increased diastolic stiffness.\textsuperscript{14} We cannot be certain, however, that [ADP] increased in this demand ischemia model, because it did not increase significantly in other studies of low-flow ischemia from our laboratory.\textsuperscript{24} Nevertheless, even severe ATP depletion occurring in only a small group of myocytes would be undetected by measurements of total tissue [ATP]. During demand ischemia, a population of more severely energy-deprived myocytes vulnerable to rigor may be interspersed among normally contracting cells. Experiments in isolated myocytes have consistently demonstrated inexcitability and contractile failure at the time of rigor shortening.\textsuperscript{25}

Thus, in the isolated heart undergoing demand ischemia, the continued development of phasic contractile force when diastolic pressure is elevated is consistent with the idea that some myocytes are not in a rigor state and are capable of contracting, whereas others are in a rigor state and are inexorable. In isolated hearts, cell-by-cell electron microscopy revealed a highly heterogeneous distribution of development of ischemic contracture when diastolic chamber stiffness had increased during low-flow ischemia.\textsuperscript{26} The extent of diastolic chamber stiffness increase may be related to the number of myocytes in rigor, which may progressively increase with continued demand ischemia (Figure 1). Contracture may be reversible, as demonstrated here when the supply-demand mismatch was corrected and as observed during reoxygenation of anoxic myocytes.\textsuperscript{22}

### Characteristics of the Model

Our experimental preparation confers many advantages for modeling demand ischemia. The right ventricle is decompressed and the pericardium freed, which eliminates interactions with the LV. The heart is subjected to global underperfusion, which prevents the confounding mechanical influence of dysynchronous contraction of ischemic and nonischemic segments associated with regional ischemia. The isovolumic preparation allows the use of QSR as an investigative tool. Use of a red-cell perfusate at 37°C containing glucose (5.5 mmol/L) and free fatty acid at a normal ratio of FFA to albumin provides normal levels of the major myocardial substrates and ensures a normal rate of oxygen delivery at physiological coronary flow rates. Elucidation of diastolic dysfunction during demand ischemia in isolated heart appears to require a critical interplay and relationship between coronary flow, energy supply, and energy demand. For example, we have found it impossible to reproduce demand ischemia–induced increases in LVEDP in hearts perfused with crystalloid solutions or at temperatures <37°C.

In summary, in this model of demand ischemia in the isolated heart in which we simulated features of diastolic anginal physiology, responses to quick length changes supported a mechanism of a reversible rigor-like tension underlying increased diastolic chamber stiffness and not a calcium-driven force.

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### References


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