Pacing-Induced Dys-Synchrony Preconditions Rabbit Myocardium Against Ischemia/Reperfusion Injury

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Background—Because increased mechanical load induces preconditioning (PC) and dys-synchrony increases loading in late-activated regions, we investigated whether dys-synchrony induced by ventricular pacing (VP) at normal heart rate leads to cardioprotection.

Methods and Results—Isolated working rabbit hearts were subjected to 35 minutes of global ischemia and 2 hours of reperfusion. Seven hearts underwent VP PC (3 periods of 5 minutes VP at the posterior left ventricular [LV] wall), 7 hearts underwent ischemic preconditioning (IPC) (3 periods of 5 minutes of global ischemia), and 9 hearts served as control (C). LV pressure and sonomicrometry were used to assess global hemodynamics and segment work (SW) and end-diastolic segment length (EDSL) in anterior and posterior LV myocardium. Myocardial release of lactate and expression of proBNP mRNA were determined to gain insight in molecular processes involved in VP PC (*P/H110210.05).

Infarct size (triphenyl tetrazolium chloride staining) was 18.3+/−13.0% in group C, and was uniformly reduced in the VP PC and IPC groups (1.8+/−0.8%, and 3.5+/−3.1%, respectively; and not significant between VP PC and IPC). LV posterior wall pacing (VP PC group) increased EDSL (by 6.3+/−5.8%*) and SW (to 335+/−207%*) in the LV anterior wall, whereas posterior wall SW decreased to negative values (−23+/−63%*). LV pacing did not significantly change lactate release and coronary flow but significantly increased proBNP mRNA expression in both anterior and posterior myocardium as compared with controls.

Conclusions—Intermittent dys-synchrony is equally cardioprotective as “classical” IPC. Stretch-mediated signaling is a more likely trigger for VP PC than ischemia. VP PC is potentially applicable in cardiac surgery. (Circulation. 2006; 114[suppl I]:I-264–I-269.)

Key Words: dys-synchrony ■ pacing ■ preconditioning ■ stretch ■ ventricular

Myocardium can be protected from the detrimental effects of ischemia and reperfusion by subjecting it to various stimuli prior to prolonged ischemia. This so-called preconditioning (PC) has been achieved by, among others, brief ischemia,1 rapid pacing,2,3 and increased cardiac preload,4,5 and afterload.6 Dys-synchronous ventricular activation, as elicited by ventricular pacing (VP), induces regional differences in myocardial mechanical work within the left ventricular (LV) wall. Early-activated regions shorten considerably during early systole, thereby stretching late-activated myocardium. As a consequence, mechanical load is increased in the latter regions.7,8

We hypothesized that the abnormal regional mechanics caused by dys-synchronous activation can induce myocardial preconditioning. This hypothesis was explored in isolated ejecting rabbit hearts by investigating the protective potency of intermittent LV pacing at normal heart rate, applied before global ischemia and subsequent reperfusion. The cardioprotective potency of ventricular pacing was compared with that of classical ischemic preconditioning and potential mechanisms were explored.

Materials and Methods
Animal handling and treatment were according to the Dutch law on animal experimentation (WOD). The protocol was approved by the animal experimental committee of the Maastricht University.

Female White New Zealand rabbits (2.5 to 3.5 kg; House-bred, Manstricht University; The Netherlands) were sedated with Hypnorm® (fluanisone 10 mg/mL and fentanyl 0.2 mg/mL, subcutaneously 0.5 mL/kg) and anticoagulated with intravenous heparin (1500 IU). After cervical dislocation the heart was quickly removed, immersed in ice-cold perfusion buffer for removal of noncardiac tissue, and attached to the perfusion apparatus.

Experimental Setup
The perfusion system was a modified Langendorff setup for the ejecting rabbit heart. The perfusate consisted of (mmol/L): sodium (153.9), potassium (5.3), calcium (2.2), magnesium (1.1),
Signals were analyzed off-line. Hemodynamic data consisted of aortic and LV pressure and aortic flow (in-line aortic flow probe). Cardiac output was calculated as the sum of coronary and aortic flow. The area of the pressure–segment length loop was calculated and used as a measure of regional segment work in anterior and posterior LV myocardium. End-diastolic segment length (EDSL) was derived from the PL-loop to assess regional stretch.

Coronary effluent was collected for determination of lactate release. Samples were immediately frozen in liquid nitrogen and stored at −80°C. Lactate was determined by spectrophotometry using a Cobas Bio autoanalyzer (Roche Diagnostics, Almere, the Netherlands). After 2 hours of reperfusion, the hearts were cut into 2-mm-thick transverse slices, which were stained with 1% triphenyl tetrazolium chloride (TTC) and fixated in 4% formaldehyde. Infarct size was quantified 24 hours later using Leica Qwin software (Leica, Rijswijk, the Netherlands). Digitally acquired images of all slices were divided into 4 LV segments (anterior, lateral, posterior, and septal) and all infarcted tissue was manually indicated on the image. Infarct size was expressed as percentage of the entire LV mass, because global ischemia was used.

Total RNA from the anterior and posterior region was extracted with TRIzol Reagent (Sigma, Zwijndrecht, the Netherlands) according to the manufacturer’s protocol. RNA samples were dissolved in RNAase-free water. The OD260/280 ratio was controlled and visual inspection of the denaturing gels was made. Total RNA (500 ng) was used for DNase treatment (Sigma) and subsequent reverse transcription (iScript cDNA synthesis kit; Biorad Inc, Hercules, Calif). Gene expression analysis in the obtained cDNA was performed by quantitative polymerase chain reaction on an iCycler real-time polymerase chain reaction detection system using the iQ SYBR Green supermix (Biorad). The following oligonucleotide primers for rabbit proBNP mRNA were used: sense primer, 5′-GTCTCCGGAAACAGGTCTCCT-3′; and antisense primer, 5′-TCTCTGGTCCTTGGTCTC-3′. The sequence of proBNP mRNA was a kind gift from A. A. Proctor, Scios Inc, Sunnyvale, Calif. All transcripts were normalized to hypoxanthine phosphoribosyltransferase and cyclophilin.

**Data Analysis**
Statistical analysis was performed on absolute values, even when presented relative to baseline (Table). ProBNP mRNA data were analyzed using a 2-sided t test. Other data were evaluated by 2-way analysis of variance for repeated measures within each group (to compare different time points during the experiment or different segments after TTC staining) and between the groups. In case of a significant difference, post-hoc analysis with Tukey correction was used for further comparison. P<0.05 was considered statistically significant. Mean values±SD are presented. The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

**Results**

**Infarct Size**
TTC staining demonstrated a significantly smaller global infarct size in the VP PC group (18.3±3.1%) than in the control group (18.3±13.0%). There was no significant difference in infarct size between the VP PC and the IPC groups (Figure 2). In the VP PC and IPC groups, infarct size was equally small in all segments and significantly smaller in each segment as compared with the corresponding segment in the control group (Figure 2).

**Hemodynamic Function**
The Table shows hemodynamic data for all groups during the entire protocol. LV pacing (VP PC group) significantly decreased LVdP/dt max; all other hemodynamic parameters

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**Figure 1.** Experimental protocol for the 3 study groups. Index ischemia was 35 minutes of global ischemia. Small white blocks: 5 minutes of left ventricular (LV) pacing at 240 bpm; small black blocks: 5 minutes of global ischemia. Right atrial (RA) pacing (indicated by the gray line) at 240 bpm was performed throughout the experiment, except during ischemia and the first 10 minutes of reperfusion after index ischemia. Arrows indicate the end of the additional experiments for analysis of proBNP mRNA. PC indicates preconditioning stimulus.

**Study Protocol and Groups**
Animals were divided into 3 study groups. In the control group (n=9), a 50-minute period preceded the index ischemia (Figure 1). In the VP PC group (n=7), LV pacing was performed during 3 5-minute periods, interspersed with 5 minutes of RA pacing. Like the VP PC group (n=7), a 50-minute period preceded the index ischemia (Figure 1). In the IPC group, LV pacing was performed during 3 5-minute periods, interspersed with 5 minutes of RA pacing. Like the VP PC group (n=7), LV pacing was performed during 3 5-minute periods of global ischemia, and heart rate at 240 bpm (physiological heart rate for rabbits) using RA pacing.

**Data, Effluent, and Tissue Collection and Processing**
Hemodynamic, electrophysiological and sonomicrometry data were recorded on the Sonometrics system at a sampling rate of 500 Hz.
remained essentially unaltered. After the IPC stimulus, LVdP/dtmax and LVdP/dtmin remained significantly depressed.

After the index ischemia cardiac output, LVPmax, LVdP/dtmax, and LVdP/dtmin remained significantly lower than at baseline in each group and postischemic values were not significantly different between the groups (Table).

Coronary Flow

LV pacing (VP PC group) did not significantly change coronary flow as compared with RA pacing (Figure 3, upper panel). After each IPC stimulus, coronary flow returned to baseline values. After index ischemia coronary flow decreased significantly below baseline level in the control (at 60 and 120 minutes) and IPC group (at 120 minutes), but not within the VP PC group. However, coronary flow was not significantly different between the groups at corresponding time points.

Lactate Release and proBNP mRNA Expression

LV pacing (VP PC group) did not significantly change lactate release (Figure 3, lower panel), whereas after each IPC stimulus lactate release increased ~6-fold. After index ische-
mia lactate release peaked within the first 5 minutes of reperfusion, with values not significantly different between the three groups (Figure 3, lower panel).

In the additional experiments it was found that proBNP mRNA was 3- to 4-fold upregulated as compared with control (P<0.05) in both the anterior and posterior LV wall 10 minutes after the third VP PC period (Figure 4).

Electrical and Mechanical Dys-Synchrony During LV Pacing
QRS duration was ≈25 ms during RA pacing and increased significantly to 48.5±5.1 ms during LV pacing. During LV pacing, segment work (pressure-length loop area) in the late-activated anterior LV wall increased significantly, whereas segment work decreased to negative values in the early-activated posterior segment (Figures 5 and 6). In the latter region segment work became negative because of early systolic shortening and late systolic stretch (Figure 5). LV posterior wall pacing significantly increased EDSL in the anterior segment (Figure 7) without significantly changing EDSL in the posterior segment (data not shown).

Discussion
The present study proves the concept that brief periods of cardiac dys-synchrony, induced by ventricular pacing at physiological heart rate, can reduce myocardial infarct size as much as classical ischemic preconditioning. The processes leading to dys-synchrony–induced preconditioning are more likely to be initiated by stretch than by ischemia.

Intermittent Dys-Synchrony as a Preconditioning Trigger
In the present experimental setting, the cardioprotection achieved by dys-synchrony is obtained while keeping heart rate, preload, and afterload constant and with only moderate reduction in contractility. Also myocardial ischemia is not a likely trigger for dys-synchrony–induced preconditioning. After all, during LV pacing coronary flow and lactate release did not change. Although lactate release from the whole heart was measured, regional ischemia is unlikely. Because no lactate is present in the perfusion buffer, any lactate in the effluent indicates myocardial release and regional lactate release cannot be concealed by lactate uptake in other regions. Moreover, previous studies in our group have shown that ventricular pacing causes a redistribution of mechanical work within the LV wall, but local lactate and oxygen extraction measurements did not show any sign of underperfusion in any region.11
A more likely trigger for dys-synchrony–induced preconditioning is the increased mechanical loading occurring in regions remote from the pacing site. Other studies already showed that increasing global load leads to preconditioning.\(^4\)

The early systolic prestretch in late-activated regions has been shown to be sufficient to lead to local hypertrophy during chronic ventricular pacing.\(^12\) Moreover, preliminary observations in 2 additional rabbit hearts, frozen immediately after the third VP PC period, show that ERK1/2 phosphorylation was increased 3- to 4-fold in the anterior, but not in the posterior, LV wall as compared with 4 control hearts. ERK1/2 is known to be activated by stretch\(^13\) and is also a well-known player in ischemic preconditioning.\(^14\)

ProBNP mRNA expression is also increased because of stretch.\(^15\) Moreover, BNP is cardioprotective when administered to the heart.\(^16\) The increased proBNP mRNA expression as well as protection throughout the LV wall is remarkable, because the mechanical trigger presumably originates in the late-activated region. In this respect it is important to note that changes in the general loading conditions in our preparation are excluded as a cause of proBNP mRNA upregulation. Protection in myocardium remote from a triggered zone is referred to as “remote preconditioning” and is known to occur in regions distant from a previously preconditioned region.\(^14,17\)

The results from the present study do not offer conclusive evidence about a causal relation between proBNP mRNA expression, ERK1/2 phosphorylation, and dys-synchrony–induced cardioprotection, and certainly do not exclude other players. Clearly, further studies are required to investigate the underlying mechanisms of dys-synchrony–induced preconditioning.

**Pacing Preconditioning: Heart Rate or Dys-Synchrony?**

In the past, several studies have shown the cardioprotective effects of rapid ventricular pacing.\(^2,3,18\) Except by 1 group of investigators,\(^18\) protection by rapid ventricular pacing has been attributed to concomitant myocardial ischemia. Interestingly, rapid atrial pacing does not induce preconditioning.\(^19\) even though coronary flow, hemodynamics, and electrophysiological variables were affected to a similar degree as during rapid ventricular pacing. With hindsight, these publications support our hypothesis that dys-synchrony rather than high heart rate induces preconditioning.

A cardioprotective effect of intermittent dys-synchrony has also been found in isolated chick embryo hearts. Intermittent ventricular pacing for 12 hours reduced the incidence of arrhythmias after ischemia.\(^20\) In the present study, we followed a “classical” preconditioning scheme in mammalian hearts and used infarct size as the principal end point.

**Potential Clinical Application**

The experimental setup of global myocardial ischemia is comparable to the situation during cardiac surgery. Under these circumstances, pacing leads are often attached to the heart for back-up pacing in case of accidental AV block. Results from preconditioning studies in isolated rabbit hearts have been shown to apply well to in vivo situations in other species, including humans.\(^14\) Therefore, it seems worthwhile and feasible to explore the protective effects of intermittent ventricular pacing in cardiac surgery.

Dys-synchrony–induced preconditioning is easier to apply and less counterintuitive than ischemic preconditioning. Its value as compared with administration of drugs, like anesthetics,\(^21\) needs to be determined. This also holds for the possibility to combine dys-synchrony–induced preconditioning with other triggers to increase the protective effect, as shown for combining ischemic preconditioning with drug administration.\(^22\)

**Conclusion**

Intermittent dys-synchrony, induced by ventricular pacing at physiological heart rate, induces uniform preconditioning in rabbit hearts. The observed cardioprotective effect is unlikely to be triggered by ischemia. Ventricular pacing preconditioning is clinically feasible and potentially useful in cardiac surgery.

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**Disclosures**

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