Phenotypic adaptation of the late preconditioned heart: Myocardial oxygen consumption is reduced

Xavier Monnet a,b,c, Bijan Ghaleh a,b,c,d, Laurence Lucat a,b,c, Patrice Colin a,b,c, Roland Zini a,b,c, Luc Hittinger a,b,c,d, Alain Berdeaux a,b,c,d,*

a INSERM U660, Créteil, F-94010, France
b École Nationale Vétérinaire d’Alfort, Maisons-Alfort, F-94700, France
c Laboratoire de Pharmacologie, Faculté de Médecine de Créteil, Université Paris XII, Créteil, F-94000, France
d Fédération de Cardiologie, Hôpital Henri Mondor, Créteil, France

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Abstract

Objectives: Although the signalling pathways of late preconditioning have been extensively investigated, its consequence for myocardial metabolism remains unknown. Thus, myocardial oxygen consumption (MVO2) was evaluated before and under late preconditioning.

Methods: In 7 chronically instrumented dogs, we measured MVO2 in vivo at baseline and during inotropic stimulation with dobutamine (10 and 20 μg/kg/min, i.v.) before (Day 0) and 24 h after (Day 1) a 10-min circumflex coronary artery occlusion.

Results: At Day 0, dobutamine dose-dependently increased the triple product (heart rate × left ventricular systolic pressure × left ventricular maximum dP/dt), MVO2, coronary blood flow, and coronary sinus pO2. At Day 1, the triple product was similar at baseline and at each dose of dobutamine but MVO2 was significantly blunted as compared to Day 0 (15±4%, 22±3% and 19±4% at baseline, dobutamine 10 and 20 μg/kg/min, respectively). Importantly, the relationship between MVO2 and triple product was significantly rightward shifted with late preconditioning, i.e., MVO2 was reduced for any level of triple product. At Day 1, the relationship between coronary blood flow and MVO2 was not altered as compared to Day 0 but coronary sinus pO2 was significantly increased vs. Day 0 for any level of coronary blood flow, suggesting that late preconditioning exerted no vasomotor effect but rather changed myocardial oxygen handling. These effects were abolished by administration of S-methyl-isothiourea (1.5 mg/kg, i.v.), an iNOS inhibitor.

Conclusion: This study demonstrates that ischemic late preconditioning is characterized by a major reduction in MVO2, both at baseline and under inotropic stimulation. NO from iNOS contributes to this modification of metabolic phenotype.

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Keywords: Oxygen consumption; Stunning; Nitric oxide; Preconditioning

1. Introduction

Late preconditioning is a cardioprotective phenomenon which develops 24 h after a brief ischemic episode leading to a reduction in infarct size [23] as well as in the severity and duration of myocardial stunning [32,34]. Numerous mechanisms have been proposed to explain the protective effect of late preconditioning [42]. Indeed, increased heat shock proteins such as hsp 27 [9], hsp 70 [21], hsp 72 [20] as well as enhanced MnSOD activity [13,41] have been proposed as possible mechanisms for this protection. It is also now well demonstrated that nitric oxide (NO) plays a major role as a trigger and mediator of late preconditioning [4]. Different isoforms of NO synthase (NOS) might be involved in this phenomenon [14,16,40,41] and iNOS was demonstrated to be upregulated during the second window of preconditioning in both rabbits [14,41] and dogs [15].
Although the signaling pathways leading to late preconditioning have been extensively investigated during the past years, little is known about the functional implications of this increase in NO production. In this setting, Kim et al. [15] have reported an enhanced iNOS activity in the contraction of myocytes isolated from late preconditioned dog hearts. However, the consequences of NOS upregulation on myocardial metabolism of the late preconditioned heart have never been yet investigated. Indeed, evidence for a role of NO on mitochondrial function has been reported since 1982 [11] and NO has been demonstrated to reduce oxygen consumption in various physiological as well as pathological conditions in the heart [1,2,5,27,28,31,37] as well as in other organs [17,30,31]. Indeed during classical ischemic preconditioning, a reduction in myocardial metabolism assessed by a preservation of ATP [22] as well as a decrease in both energy demand [18] and myocardial oxygen consumption [35] have already been demonstrated. Whether myocardial oxygen consumption is altered during the late phase of preconditioning and whether such alteration is a feature of this cardioprotection has never been studied yet.

Therefore, the first goal of our study was to determine whether myocardial oxygen consumption (MVO₂) is modified 24 h after a brief coronary artery occlusion (CAO). For this purpose, we used a model of chronically instrumented conscious dogs that enables in vivo to repeat CAO 24 h apart and to extensively monitor hemodynamic and metabolic parameters [7,10]. MVO₂ was measured at baseline and during inotropic stimulation with graded doses of dobutamine. The potential modulatory role of NO/iNOS on MVO₂ was investigated by administrating S-methyl-isothiourea (SMT), an iNOS inhibitor [3,24].

2. Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1996).

2.1. Instrumentation

Seven dogs (17–24 kg) were anesthetized with pentobarbital sodium (30 mg/kg i.v.), intubated and mechanically ventilated with a Harvard respirator (South Natick, MA, USA). The anesthesia was maintained with additional administrations of pentobarbital sodium. Under sterile surgical conditions, a left thoracotomy (5th intercostal space) was performed. The heart was suspended in a pericardial cradle. Fluid-filled Tygon catheters were placed in the descending thoracic aorta and the left atrium for measurements of blood pressures. Silastic catheters were implanted in the pulmonary artery for drug administration and in the coronary sinus with the tip leading away from the right atrium for venous blood sampling. A solid-state pressure transducer (P7A, Konigsberg Instruments; Pasadena, CA, USA) was introduced into the apex of the left ventricle (LV). A coronary flow probe (Transonic Systems; Ithaca, NY, USA) and a pneumatic occluder were placed on the left circumflex coronary artery. Two pairs of ultrasonic crystals were used for measurement of left ventricular (LV) wall thickening in the distribution of the left circumflex (posterior zone) and the left anterior descending (anterior zone) coronary arteries. One crystal was implanted in the endocardium and the other was sutured to the epicardium. Finally, bipolar electrodes were sewn on the left atrial appendage for subsequent pacing. All catheters and wires were exteriorized between the scapulae, and the pneumothorax was evacuated through a chest tube inserted in the sixth intercostals space. Cefazolin (1 g i.v.) and gentamicin (40 mg i.v.) were administered before and during the first week after surgery. Post-operative analgesia was provided with morphine and buprenorphine.

2.2. Hemodynamic measurements

All hemodynamic data were recorded and analyzed using the data acquisition software HEM v3.3 (Notocord Systems; Croissy sur Seine, France). Aortic and left atrial pressures were measured with a Statham P23 ID strain-gauge transducer (Gould-Nicolet; Courttaboeuf, France). Mean coronary blood flow (CBF) was measured using a transit-time flowmeter (Transonic T206, Transonic Systems; Ithaca, NY, USA). LV pressure was measured using the Konigsberg gauge and the change in LV pressure over time (LV dP/dt) was computed from the LV pressure signal. LV pressure was calibrated in vitro with a mercury manometer and in vivo with the left atrial and aortic pressures. Cardiac work was estimated by the triple product as heart rate × systolic arterial pressure × LV dP/dtmax.

2.3. Measurements of LV wall thickness

Wall thicknesses were obtained by using an ultrasonic transit-time dimension gauge (Module 201, System 6, Triton Technology Inc.; San Diego, CA, USA). To determine wall thickening, end-diastolic wall thickness was measured at the initiation of the upstroke of LV pressure tracing and the end-systolic wall thickness was measured 20 ms before peak negative LV dP/dt. Percent wall thickening was defined as end-systolic thickness minus end-diastolic thickness times 100 divided by end-diastolic thickness.

2.4. LV oxygen consumption

Measurement of oxymetric parameters was made with a blood gas apparatus and a co-oxymeter. Oxygen consumption was calculated as the product of CBF and the arteriovenous difference in the oxygen content. The arteriovenous difference in oxygen content was measured
between the arterial oxygen content and the coronary sinus oxygen content.

2.5. Myocardial nitrate–nitrite measurements

Blood samples obtained from the aortic and the coronary sinus catheters for determination of NOx (total plasma nitrite and nitrate concentrations) were collected into heparinized syringes. After centrifugation, plasma NOx was determined using a fluorometric assay (Cayman fluorometric nitrate–nitrite assay kit, Ann Arbor, MI, USA). The coronary arteriovenous difference of NOx was obtained by subtracting coronary venous from aortic NOx. The coronary production of NOx (MNOx, nmol/l) was calculated by multiplying the arteriovenous difference by the corresponding mean coronary blood flow.

2.6. Experimental protocol

The experiments were conducted 3–4 weeks after surgery when the dogs were healthy and apyretic. At Day 0, when dogs were lying quietly on a table, a first set of hemodynamic measurements was performed and a first blood sample was collected for blood gas analysis. Dobutamine was then infused i.v. continuously during 5 min at 10 μg/kg/min. Hemodynamic and oxymetric parameters were measured once hemodynamic stability was achieved. The rate of dobutamine infusion was then increased at 20 μg/kg/min during 5 min and a third set of measurements was recorded at stability. Another day, a 10-min circumflex coronary artery occlusion (CAO) was performed by complete inflation of the pneumatic occluder.

Twenty-four hours after CAO (Day 1), hemodynamic and oxymetric parameters were measured at baseline and during dobutamine infusion (10 and 20 μg/kg/min during 5 min). When hemodynamics had returned to baseline values, SMT (S-methyl-isothiourea) was administered (1.5 mg/kg, iv). Thirty minutes later, infusion of dobutamine (10 and 20 μg/kg/min during 5 min) associated with hemodynamic and oxymetric measurements was repeated again. In order to strictly control heart rate for MVO2 measurements and to avoid the confounding effect of potential slight variations in heart rate [7], all measurements at Day 0 and Day 1 before SMT as well as at Day 1 after SMT were performed under atrial pacing (spontaneous heart rate at baseline and under dobutamine observed at Day 0 ± 10 beats/min), i.e., comparisons of MVO2 values within each animal were performed at the same heart rate.

The selected dose of SMT was based on preliminary experiments performed in additional 4 dogs showing that it was the highest one that did not induce any significant hemodynamic changes. Furthermore, we tested that the CBF responses to acetylcholine (0.3 to 3 μg/kg, i.v.) were similar in the absence and presence of SMT. In addition, MVO2 measured at baseline and during dobutamine infusion (10 and 20 μg/kg/min during 5 min) were not altered by SMT.

This is illustrated in Fig. 1 showing the absence of changes in the relationship between MVO2 and the triple product by SMT in the non-preconditioned state.

To ensure that late preconditioning against myocardial stunning was effective in each investigated animal, 10 days later, 2 episodes of 10-min CAO were performed 24 h apart and hemodynamic was recorded at spontaneous heart rate before CAO, during CAO and during the ensuing reperfusion period (6h).

2.7. Statistical analysis

Data are reported as mean ± S.E.M. Comparisons were performed using two-way ANOVA for repeated measures. If needed, individual comparisons were then conducted using a paired Student’s t-test with Bonferroni correction. In addition, linear regressions were performed and analysis of covariances were used to adjust a response variable for linear dependence on an investigated parameter after testing for parallel regression lines. A value of p < 0.05 was considered significant.

3. Results

3.1. Hemodynamics

Hemodynamic data are summarized in Table 1. At Day 0, dobutamine dose-dependently increased the triple product to 21.3 ± 2.3 and 33.3 ± 3.6 \(10^5\) beats/mm Hg\(^2\)/s\(^2\) from 10.8 ± 1.3 \(10^5\) beats/mm Hg\(^2\)/s\(^2\) at 10 and 20 μg/kg/min, respectively. Wall thickening in the posterior zone was also increased to 51 ± 8% and 54 ± 8% from 35 ± 7% with dobutamine at 10 and 20 μg/kg/min, respectively. At Day
Day 0

Table 1

Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Base</th>
<th>Dobu 10</th>
<th>Dobu 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>113±4</td>
<td>116±4</td>
<td>149±4</td>
</tr>
<tr>
<td>Day 1</td>
<td>115±4</td>
<td>123±6</td>
<td>150±6</td>
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<tr>
<td>Day 1 + SMT</td>
<td>114±5</td>
<td>118±4</td>
<td>147±7</td>
</tr>
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<td>Left ventricular pressure (mm Hg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>142±7</td>
<td>165±7</td>
<td>176±7</td>
</tr>
<tr>
<td>Day 1</td>
<td>138±4</td>
<td>160±6</td>
<td>172±7</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>142±3</td>
<td>169±7</td>
<td>180±6</td>
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<tr>
<td>Left ventricular dP/dt (mm Hg/s)</td>
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<td>4190±296</td>
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<td>10053±676</td>
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<td>3971±335</td>
<td>7303±567</td>
<td>9559±671</td>
</tr>
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<td>3933±367</td>
<td>7275±450</td>
<td>10007±493</td>
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<td>Triple product (beats/mm Hg g/cm²)</td>
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<tr>
<td>Day 0</td>
<td>10.8±1.3</td>
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<td>33.3±3.6</td>
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<tr>
<td>Day 1</td>
<td>9.8±1.1</td>
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<td>35.2±2.9</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>10.0±1.1</td>
<td>21.7±1.9</td>
<td>33.3±3.4</td>
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<td>Posterior wall thickening (%)</td>
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<tr>
<td>Day 0</td>
<td>35±7</td>
<td>51±8</td>
<td>54±8</td>
</tr>
<tr>
<td>Day 1</td>
<td>33±6</td>
<td>50±8</td>
<td>53±8</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>34±8</td>
<td>50±9</td>
<td>56±9</td>
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<tr>
<td>Posterior end-diastolic wall thickness (mm)</td>
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<td>8.4±0.1</td>
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<td>Day 1</td>
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<tr>
<td>Day 1 + SMT</td>
<td>8.2±0.5</td>
<td>8.2±0.5</td>
<td>8.4±0.5</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M., n=7.

Base: baseline.
Dobu 10: dobutamine infusion at 10 µg/kg/min.
Dobu 20: dobutamine infusion at 20 µg/kg/min.
SMT: S-methyl-isothiourea.

Table 2

Coronary blood flow and oximetric parameters

<table>
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<tr>
<th></th>
<th>Base</th>
<th>Dobu 10</th>
<th>Dobu 20</th>
</tr>
</thead>
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<tr>
<td>CBF (ml/min)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>35±2</td>
<td>58±6</td>
<td>76±9</td>
</tr>
<tr>
<td>Day 1</td>
<td>32±2</td>
<td>50±6*</td>
<td>65±8*</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>35±3</td>
<td>54±5</td>
<td>70±7</td>
</tr>
<tr>
<td>pO2 (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>20.6±1.1</td>
<td>25.0±0.8</td>
<td>26.4±0.8</td>
</tr>
<tr>
<td>Day 1</td>
<td>21.7±2.0</td>
<td>27.6±1.9</td>
<td>27.9±2.4</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>19.9±1.8</td>
<td>24.6±1.8</td>
<td>26.0±2.4</td>
</tr>
<tr>
<td>pO2 (mm Hg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>101.1±1.4</td>
<td>101.6±2.0</td>
<td>101.6±3.0</td>
</tr>
<tr>
<td>Day 1</td>
<td>102.3±3.1</td>
<td>106.4±2.4</td>
<td>105.6±2.7</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>105.0±3.1</td>
<td>104.3±2.3</td>
<td>105.6±2.6</td>
</tr>
<tr>
<td>C02O2 (ml/100 ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>4.7±0.2</td>
<td>7.3±0.5</td>
<td>7.8±0.4</td>
</tr>
<tr>
<td>Day 1</td>
<td>5.3±0.5</td>
<td>8.4±0.6</td>
<td>8.5±0.8</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>4.3±0.4</td>
<td>6.9±0.7</td>
<td>7.7±0.9</td>
</tr>
<tr>
<td>C02O2 (ml/100 ml)</td>
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<td></td>
<td></td>
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<tr>
<td>Day 0</td>
<td>17.9±0.9</td>
<td>20.1±0.8</td>
<td>21.3±0.7</td>
</tr>
<tr>
<td>Day 1</td>
<td>17.4±0.7</td>
<td>19.6±1.9</td>
<td>20.3±0.7</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>15.9±0.9</td>
<td>19.2±1.8</td>
<td>20.2±0.6</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>13.5±0.7</td>
<td>15.2±0.7</td>
<td>16.2±0.5</td>
</tr>
<tr>
<td>Day 1</td>
<td>13.1±0.5</td>
<td>14.8±0.6</td>
<td>15.3±0.6</td>
</tr>
<tr>
<td>Day 1 + SMT</td>
<td>11.9±0.7</td>
<td>14.5±0.5</td>
<td>15.3±0.5</td>
</tr>
</tbody>
</table>

Values are mean±S.E.M., n=7.

Base: baseline.
Dobu 10: dobutamine infusion at 10 µg/kg/min.
Dobu 20: dobutamine infusion at 20 µg/kg/min.
SMT: S-methyl-isothiourea.
C02O2: O2 content of the coronary sinus; C02O2: arterial O2 content.
*p < 0.05 vs. Day 0.

Fig. 2 and Table 2 show the CBF and oximetric parameters. At Day 0, dobutamine infusion dose-dependently increased MVO2 to 7.6±0.8 and 9.5±1.1 ml O2/min from 4.7±0.3 ml O2/min at 10 and 20 µg/kg/min, respectively. CBF also increased from 35.2±ml/min at baseline to 58.6±ml/min and 76.9±ml/min at 10 and 20 µg/kg/min of dobutamine, respectively. Values of pO2 were simultaneously increased under dobutamine.

At Day 1, baseline MVO2 was significantly reduced vs. Day 0 (4.1±0.5 vs. 4.7±0.3 ml O2/min, respectively). In addition, production of NOx was increased at Day 1 as compared to Day 0 (23.4± vs. 12.3± nmol/min, respectively, p < 0.05). During dobutamine infusion, MVO2 increased to 6.0±0.7 and 7.7±0.8 ml O2/min from baseline at 10 and 20 µg/kg/min, respectively. These effects of dobutamine on MVO2 observed at Day 1 were however of lower magnitude as compared to corresponding ones at Day 0 (−22±3% and −19±4% at 10 and 20 µg/kg/min, respectively) (Fig. 2). Simultaneously, the dobutamine-induced increases in CBF were significantly reduced under dobutamine at Day 1 vs.
Day 0 (−13±4% and −14±3% at 10 and 20 µg/kg/min, respectively) (Table 2). These effects of late preconditioning on CBF and oxymetric parameters were abolished after SMT administration (Table 2; Fig. 2). Values of hemoglobin and \( \text{pH} \) were not significantly different between Day 0, Day 1 without SMT and Day 1 plus SMT.

When MVO\(_2\) was plotted against the triple product (Fig. 3), analysis of covariance revealed that MVO\(_2\) was significantly reduced at Day 1 as compared to Day 0 for any level of the triple product. Administration of SMT abolished this effect. *\( p < 0.05\) vs. Day 0.

Finally, analysis of covariance demonstrated that pcsO\(_2\) values measured at Day 1 were significantly higher than those measured at Day 0 for any level of CBF. This effect was abolished by the administration of SMT as illustrated in Fig. 4.

### 3.3. Late preconditioning against myocardial stunning

At Day 0, CAO induced a dramatic decrease of LV wall thickening in the posterior zone (to −1 ± 2% from 40±8%) that progressively returned to its baseline value within 24 h. At Day 1, i.e., 24 h later, CAO induced a similar decrease in LV wall thickening but the recovery of this parameter was significantly greater during reperfusion vs. Day 0, demonstrating late preconditioning against myocardial stunning (Fig. 5).

### 4. Discussion

This study conducted in chronically instrumented conscious dogs demonstrates that (a) in the late preconditioned state, i.e., 24 h after a brief coronary occlusion, MVO\(_2\) is reduced at baseline as well as during an inotropic stimulation for a similar myocardial demand, (b) this reduction in MVO\(_2\) is related to a change in oxygen utilization and (c) NO related to iNOS contributes to this adaptation of the cardiac metabolic phenotype during late preconditioning as it is reversed by the administration of SMT. These results provide the first evidence that ischemic late preconditioning induces profound alterations in myocardial oxygen handling in a highly integrated experimental model.

The main result of this study is that in the preconditioned state, MVO\(_2\) measured at baseline and during increased oxygen demand secondary to inotropic stimulation was reduced for any level of cardiac work. Indeed, a significant rightward shift of the MVO\(_2\)–triple product relationship was observed, demonstrating that a lower amount of O\(_2\) was necessary to provide the same level of contractile performance, thus suggesting an improvement in cardiac efficiency.
Cardiac work and efficiency were not directly measured in this study but calculation of the triple product is known to be a reliable surrogate for cardiac work [26]. It is unlikely that changes in stroke volume, an important correlate of MVO$_2$ [26], might explain these results as no significant hemodynamic changes were observed between Day 0 and Day 1.

In situations of increased cardiac work such as physical exertion [2,7], p$_o$O$_2$ is known to decrease while MVO$_2$ increases. There is a concomitant vasodilation and greater oxygen extraction allowing the heart to satisfy the increased oxygen demand. In our experimental conditions, dobutamine-induced increase in MVO$_2$ was accompanied by both increases in CBF and p$_o$O$_2$, related to the vasodilatory properties of dobutamine. Therefore, it should be stressed that vasodilation induced by dobutamine was beyond the sole metabolic autoregulation, allowing p$_o$O$_2$ to increase while MVO$_2$ is also increasing. Comparisons of Days 0 and 1 revealed that CBF under dobutamine was reduced and p$_o$O$_2$ was increased during late preconditioning, concomitantly with the decrease in MVO$_2$. Regarding these changes in p$_o$O$_2$, we determined in preliminary experiments that the variability of p$_o$O$_2$ measurements is as high as 4.8% in our conditions. The lack of any significant changes in the relationship between CBF and MVO$_2$ suggests that the changes in MVO$_2$ observed between Days 0 and 1 are unlikely to be the consequence of vasomotor alterations induced by late preconditioning. In addition, the relationship between p$_o$O$_2$ and CBF was significantly leftward shifted, i.e., at any level of CBF, p$_o$O$_2$ was increased at Day 1 vs. Day 0. Therefore, the reduction in MVO$_2$ observed at Day 1 was more likely related to an improved myocyte oxygen utilization rather than an enhanced oxygen delivery to the myocardium.

In this study, we further investigated whether NO was involved in the metabolic adaptation observed at Day 1 as NO is known to play a key role during late preconditioning [3] and that NO exerts an inhibitory effect on MVO$_2$ in normal hearts [1,2,27,28,31] although other studies found no effect [8,39] or an inverse effect [33]. This metabolic effect of NO has also been demonstrated in vivo during heart failure [36,37] and was related to NO produced by the inducible isoform of NO synthase [5]. Indeed, it is known that NO acts on oxygen consumption by nitrosylating the iron–sulfur centers of cytochrom C oxidase of the mitochondrial electron transport chain, of aconitase and of mitochondrial complexes I and II by competition with oxygen [6,11,12,38,39]. NO also regulates the glucose uptake of myocytes [40] and NOS inhibition leads to the switch of cardiac metabolic substrate from free fatty acids to lactate and glucose [2] with enhanced myocardial metabolic efficiency [25]. In this study, administration of SMT at Day 1 abolished the reduction in MVO$_2$ induced by late preconditioning for similar levels of cardiac demand, suggesting that NO from iNOS was involved. Although we cannot exclude, it is unlikely that eNOS was affected by SMT in our experimental conditions as in preliminary experiments (see Methods), administration of SMT did not alter heart rate, mean arterial pressure, MVO$_2$ and coronary blood flow responses to acetylcholine. Furthermore during late preconditioning, SMT did not significantly change the p$_o$O$_2$–CBF as well as the CBF–MVO$_2$ relationships as compared to Day 0. In preliminary experiments, we could observe in 2 dogs that administration of SMT was able to abolish late preconditioning against myocardial stunning (data not shown). Finally at Day 1, although the hemoglobin level was slightly but not significantly reduced after the SMT infusion at baseline, it is unlikely that this difference could explain the changes in MVO$_2$ observed with SMT. Indeed, this difference in hemoglobin was only observed at baseline but not under dobutamine infusion, although the increases in MVO$_2$ with SMT were observed both at baseline and under dobutamine. Furthermore, this difference in hemoglobin at baseline did not induce significant change in corresponding CBF.

Previous studies demonstrated that eNOS plays a major role in the physiological regulation of mitochondrial respiration or cardiac metabolism under physiological conditions [2,16,33,38]. Conversely, Chen et al. [5] demonstrated in an in vivo dog model of heart failure that the modulation of MVO$_2$ by NO was related, at least in part, to iNOS activity. In an in vitro study, Loke et al. [19] demonstrated using hearts from eNOS$^{-/-}$ and iNOS$^{-/-}$ deficient mice that eNOS was responsible for MVO$_2$ modulation in normal heart but iNOS was also implicated if mice were previously treated with endotoxin. Thus, it is interesting to speculate that the endothelial isoform of NO might be responsible for the NO-mediated MVO$_2$ modulation in the normal setting [19,29] but that the inducible isoform of NOS would be rather implicated under pathological or adaptive conditions such as endotoxic shock [19], heart failure [5] or late preconditioning. Accordingly, iNOS was demonstrated to be upregulated during the second window of preconditioning in rabbits [14,41] and in a dog model similar to that used in the present study [15]. We suggest that ischemic late preconditioning might represent another situation where NO acts as a potent regulator of MVO$_2$ although we did not specifically investigated these mechanisms at the cellular level in our in vivo model. Because other NO synthase inhibitors induce major alterations in cardiac preload and afterload that might be confounding variables for analysing MVO$_2$, it was not possible to investigate the role of eNOS in our experimental in vivo model and this is a limitation of our study.

In conclusion, this in vivo study demonstrates for the first time that ischemic late preconditioning is characterized by a major reduction in myocardial oxygen consumption both at baseline and under inotrop stimulation. This effect is more likely related to changes in oxygen handling rather than an effect on myocardial oxygen delivery. As this effect is blunted by SMT, it suggests that NO produced from iNOS contributes to this metabolic change.
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