Triggers and promoters of ischaemic preconditioning

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Abstract

Ischaemia/reperfusion injury in coronary artery disease patients confers increased morbidity and mortality due to pump dysfunction and arrhythmia. In cardiac patients, ischaemic episodes still occur in an unpredictable manner despite multiple drug therapies and revascularization strategies. Therefore, unrelenting efforts in the way of ischaemic preconditioning have been underway to devise treatment modalities for preparing the heart to face ischaemia in a more benign (ischemia can never be benign) manner.

Ischaemic preconditioning describes the process of increasing myocardial tolerance to ischaemia/reperfusion (I/R) injury by providing sub lethal episodes of ischaemia/reperfusion. Murray et al. described the preconditioning process in 1986 [1]. They have shown that brief, intermittent bouts of ischaemia followed by reperfusion had a protective effect on canine myocardial tissue against a subsequent ischaemic insult of prolonged duration. When these preconditioned animals were subjected to a 40-min occlusion of left circumflex artery, the ensuing myocardial necrosis was only 25% of those hearts not previously subjected to preconditioning. The process of preconditioning has two phases, the early preconditioning phase, which occurs in the first 3 h and the late phase, which occurs between 12 to 72 h [2] after the ischaemic insult. Several proposed mechanisms for the preconditioning response initially included: (1) decreased tissue accumulation of breakdown products of glycogen and adenine nucleotides such as lactate, H⁺, inorganic phosphate and NH₃, (2) activation or synthesis of enzyme systems to protect myocardium from ischaemic injury.

A list of triggers and mediators of the preconditioning process include, adenosine [3], acetylcholine [4], bradykinin [5], and lipopolysaccharides [6] (Tables 1 and 2). It is hypothesized that these agents mediate preconditioning through nitric oxide (NO), which is generated by activation of endothelial derived constitutive nitric oxide synthase enzyme (cNOS). Since Furchgott [7], in 1986 identified NO as the endothelial derived relaxing factor, it has been implicated in numerous cardiovascular diseases, atherosclerosis, hypertension and cardiomyopathy. Aside from the disease process, it has also been described to have key roles in normal homeostasis and protection against cellular injury [8]. Thus the seminal role of NO in ischaemic preconditioning remains an active area of research.

Lipopolysaccharides (LPS) have been shown by Brown et al. to be a trigger in the late preconditioning process [9]. Monophosphoryl lipid A (MLA), a modified nontoxic endotoxin, is a powerful trigger for the production of inducible NO synthase (iNOS). This theory has led to recent experimentations and hypotheses that late preconditioning may be mediated via the NO pathway, although the exact molecular chain of events is unknown.

In this issue of Cardiovascular Research, Wang et al. [10] have hypothesized that LPS triggers the late preconditioning process against myocardial infarction via iNOS gene expression. The investigators, using a rat model of myocardial ischaemia/reperfusion, showed that LPS pro-

See article by Wang et al. [10] (pages 33–42) in this issue.

Table 1

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Adenosine</td>
<td>Through adenosine A, receptor activation mediates PKC and tyrosine kinase</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>Protein kinase activation</td>
</tr>
<tr>
<td>Opioids i.e. morphone</td>
<td>Activation of S, ο-opioid receptor (Gi/o protein-mediated) [12]</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>η-adrenergic receptor mediated protein kinase activation</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Activation of ATP-sensitive potassium channels.</td>
</tr>
<tr>
<td>Serotonin</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cytokines, IL1, IL2</td>
<td>Induce iNOS protein expression [13].</td>
</tr>
<tr>
<td>TNF α, Interferon</td>
<td>Induce iNOS protein expression [13].</td>
</tr>
</tbody>
</table>

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Table 2
Extrinsic inducers of ischemic preconditioning

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Mechanism</th>
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<tbody>
<tr>
<td>Lipopolysaccharides (LPS)</td>
<td>– Produces Heat Shock Protein 70i (hsp 70i) expression in myocardium [14].</td>
</tr>
<tr>
<td>(bacterial endotoxin)</td>
<td>– iNOS gene induction [15].</td>
</tr>
<tr>
<td></td>
<td>– Increases expression of c-jun and c-fos mRNA’s and mRNAs encoding catalase</td>
</tr>
<tr>
<td></td>
<td>and Mn-containing superoxide dismutase</td>
</tr>
<tr>
<td>Monophosphoryl lipid (MLA)</td>
<td>– Similar to LPS</td>
</tr>
<tr>
<td>Pharmacological agents</td>
<td></td>
</tr>
<tr>
<td>Potassium channel activators i.e.</td>
<td>– Open ATP sensitive potassium channel directly</td>
</tr>
<tr>
<td>dimakalin, cromakalin, nicorandil</td>
<td></td>
</tr>
</tbody>
</table>

vided a late preconditioning protection against myocardial infarction. This protection was preceded by increased levels of iNOS mRNA and protein. Using dexamethasone as a suppressor of iNOS gene expression and aminoguanidine as a selective inhibitor of iNOS, they found the cardioprotection was abolished with dexamethasone while aminoguanidine attenuated it. Thus, they concluded that iNOS activation by LPS is essential for the development of cardioprotection in the late preconditioning process. The rise in iNOS mRNA and protein expression 4 h after LPS injection and their disappearance during the subsequent ischaemic insult suggest its role as a trigger.

An inducible isoform of NO synthase, iNOS is present in many cells of the body including myocytes, vascular smooth muscle and macrophages. It is readily activated by a number of inflammatory cytokines such as IL-1B, IL-2, IFN-γ, TNF-α, invading tumors, cardiac ischaemia and bacterial metabolites including LPS.

The exact molecular mechanism of late preconditioning remains enigmatic. However, the current study underscores the role of iNOS not only as a trigger but also a mediator of LPS induced late cardiac preconditioning. While iNOS production seems to be a crucial step in the preconditioning process, the signaling mechanisms to link it is increased activity to lethal insults still need to be worked out (Fig. 1). The increased iNOS during an insult appears to be related to a positive feedback mechanism triggered by NO and reactive oxygen species, both of which are readily formed in various tissues based on the availability of l-arginine or tetrahydrobiopterin. NO and reactive oxygen species activate the transcription factor NF-κB which in turn increases transcription of iNOS gene. NO has a potent

Myocardial ischaemia

Lipopolysaccharides

External triggers

Pharmacological agents

Adenosine

Acetyl choline

Bradykinin

Cytokines

Endogenous mediators

iNOS mRNA expression and Increased protein synthesis

K<sub>ATP</sub> channel opening

K<sub>ATP</sub> channel opening

Early preconditioning

Late preconditioning

Fig. 1. A schematic diagram depicting mechanisms of preconditioning.
biphasic effect on NF-κB activity and has the ability to up or down regulate the expression of iNOS gene [11]. Increased iNOS production has been suggested to increase both nitric oxide and free radicals thus completing the positive feed back loop. These radicals increase the transcription of iNOS.

The exact cellular mechanisms of late preconditioning remain unclear. However, it is clear that there is a temporal relationship with the increased production of iNOS during the late preconditioning phase. The subsequent NO production may be directly or indirectly beneficial.

References