Anaesthetic preconditioning but not postconditioning prevents early activation of the deleterious cardiac remodelling programme: evidence of opposing genomic responses in cardioprotection by pre- and postconditioning#

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Background. Anaesthetic preconditioning (A_PreC) and postconditioning (A_PostC) both provide protection against ischaemia–reperfusion in the heart. However, post-ischaemic gene responses may differ between the two therapeutic strategies.

Methods. Isolated perfused rat hearts were exposed to 40 min test ischaemia followed by 3 h reperfusion and used to determine transcriptional changes in response to A_PreC and A_PostC. A_PreC was induced by 15 min of isoflurane 2.1 vol% followed by 10 min of washout, and A_PostC was induced by 15 min of isoflurane 2.1 vol% administered at the onset of reperfusion. Untreated hearts served as ischaemic control (ISCH). Coupled-two way clustering (CTWC) and principal component analysis (PCA) were used to identify gene expression patterns.

Results. A_PreC (7[SD 1]%) and A_PostC (6[2]%) produced a similar decrease in infarct size (ISCH 36[1]%, P<0.05). However, post-ischaemic genomic reprogramming was completely different. Few genes were jointly regulated (2.1‰ of upregulated genes and 1.3‰ of downregulated genes). Eight stable gene clusters including three subclusters emerged from CTWC and were related to inflammation, signalling, ion channels, transcription factors, long interspersed repetitive DNA, heat shock response and remodelling. Two stable sample clusters were identified for postconditioned hearts (first cluster) and for all other protocols (second cluster), emphasizing the unique cardiac phenotype elicited by A_PostC. PCA revealed a close genomic relationship between A_PreC and non-ischaemic healthy myocardium.

Conclusions. A_PreC, but not A_PostC, induces a post-ischaemic gene expression profile similar to virgin myocardium and prevents activation of the deleterious cardiac remodelling programme. Hence A_PreC and A_PostC are not interchangeable with respect to their molecular outcome in the heart.

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the cellular response to reperfusion. For clinicians, postconditioning is particularly promising as no prior knowledge of the onset of the ischaemic event is required to provide effective protection.

An increasing body of research supports the concept that pre- and postconditioning, independent of whether ischaemia or pharmacological agents are used for triggering, have many signalling components in common, including reactive oxygen species, mitochondrial K$_{ATP}$ channels, and in particular the reperfusion injury salvage kinases. Recently, differences with respect to key signalling steps as well as genomic responses were reported between ischaemic and pharmacological preconditioning. Ischaemic as opposed to pharmacological preconditioning elicits a post-ischaemic gene expression profile more similar to unprotected myocardium, while post-ischaemic gene expression after A$_{PreC}$ is closer to non-ischaemic healthy myocardium. To date, no direct comparisons between A$_{PreC}$ and A$_{PostC}$ have been performed at the gene expression level. Therefore this study evaluated the myocardial responses to A$_{PreC}$ and A$_{PostC}$ on a genome-wide scale. In accordance with the reported comparable functional and structural protection of the two therapeutic strategies at opposite ends of ischaemia, we hypothesized that post-ischaemic gene expression would be similar in A$_{PreC}$ and A$_{PostC}$. To test this hypothesis, we used microarray technology, which revolutionized the analysis of gene expression patterns, to dissect the complex biological processes involved in anaesthetic cardioprotection.

Data presented herein provide strong evidence that the transcriptional responses to A$_{PreC}$ and A$_{PostC}$ are, in the main, completely different, supporting the concept that, despite comparable short-term functional and structural protection, the molecular outcome and (potentially) associated long-term effects may be different. In fact, important biological pathways related to post-ischaemic myocardial remodelling were regulated in an opposite manner in A$_{PreC}$ and A$_{PostC}$.

**Materials and methods**

The experimental protocols used in this investigation were approved by the Animal Care and Use Committee of the University of Zurich, and all procedures conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiological Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication 85–23, revised 1996).

**Isolated perfused rat heart preparation**

Male Wistar rats (250 g) were heparinized (500 units i.p.) and decapitated 20 min later without prior anaesthesia. The hearts were removed and perfused in a non-circulating Langendorff apparatus with Krebs–Henseleit buffer (Na$^+$ 155 mmol litre$^{-1}$, K$^+$ 5.6 mmol litre$^{-1}$, Cl$^-$ 138 mmol litre$^{-1}$, Ca$^{2+}$ 2.1 mmol litre$^{-1}$, PO$_4^{3-}$ 1.2 mmol litre$^{-1}$, HCO$_3^-$ 25 mmol litre$^{-1}$, Mg$^{2+}$ 0.56 mmol litre$^{-1}$, glucose 11 mmol litre$^{-1}$) gassed with 95% oxygen–5% carbon dioxide (pH 7.4, 37°C). The solution was filtered through a microfibre filter (2 μm pore). The perfusion pressure was set to 80 mm Hg. A water-filled balloon was inserted into the left ventricle and inflated to set an end-diastolic pressure of 0–5 mm Hg during the initial equilibration. The distal end of the catheter was connected to a performance analyser (Plugsys Modular System, Hugo Sachs, March-Hugstetten, Germany) by means of a pressure transducer. Perfusion pressure, epicardial ECG and coronary flow (Transit Time Flowmeter Type 700, Hugo Sachs, March-Hugstetten, Germany) were recorded and processed on a PC using IsoHeart software (Hugo-Sachs, March-Hugstetten, Germany).

**Experimental protocols, analysis of cardiac function and infarct size measurements**

Spontaneously beating hearts were equilibrated for 10 min. A$_{PreC}$ was induced by isoflurane administered for 15 min at 1.5 minimum alveolar concentration (MAC) (2.1 vol%). Isoflurane was washed out for 10 min prior to 40 min of test ischaemia. The buffer solution was equilibrated with isoflurane using an Isotec 3 vaporizer (Datex-Ohmeda, Tewksbury, MA) with an air bubbler. Isoflurane concentrations were also measured in the buffer solution using a gas chromatograph (Perkin-Elmer, Norwalk, CT): isoflurane 2.1% (vol/vol) (1.5 MAC in rats at 37°C) at 0.53 (sd 0.05) mM. A$_{PostC}$ was induced by 15 min iso flurane administration at 1.5 MAC at the onset of reperfusion after 40 min of test ischaemia. Hearts subjected to ischaemia and reperfusion alone served as ischaemic controls (ISCH). All hearts were perfused for 3 h after test ischaemia. The control group (CTL) consisted of time-matched perfused hearts (270 min of perfusion). For each experimental group, five hearts were prepared and functional parameters recorded (Fig. 1). An additional five hearts in each group were used to determine infarct size using 1% triphenyltetrazolium staining as previously described. Repeated-measures analysis of variance was used to evaluate differences in functional recovery over time between groups. Unpaired t-tests were used to compare groups at identical time points, and paired t-tests to compare within groups over time (SigmaStat v 2.0; SPSS Science, Chicago, IL). The post hoc Bonferroni test was used for multiple comparisons. Corrected $P<0.05$ was considered to be statistically significant. Data are presented as means [sd].

**RNA extraction and cDNA synthesis**

The following procedures have been described in detail previously. Briefly, left ventricular tissue was frozen in liquid nitrogen and stored at −80°C. Hearts were powdered in liquid nitrogen and homogenized in TRIzol LS reagent (Invitrogen, Basel, Switzerland) and chloroform–isoamyl alcohol (Fluka, Buchs, Switzerland). The aqueous phase...
was mixed with isopropanol and precipitated overnight at 
−20°C. The pellet was washed with isopropanol, dried at 
37°C and eluted in water treated with diethyl pyrocarbonate. 
Single-strand cDNA synthesis from total RNA was performed 
using Superscript II (Invitrogen, Basel, Switzerland). The 
Superscript kit (Invitrogen, Basel, Switzerland) was used to 
synthesize double-strand cDNA. Biotin-labelled antisense 
cRNA was obtained using an RNA transcript labelling kit 
(BioArray; Enzo, Farmingdale, NY).

Hybridization and scanning of microarrays
The Affymetrix Rat Genome U34A array (Affymetrix, Santa 
Clara, CA) was used for gene expression profiling. U34A 
contains a total of 8799 probe sets representing 7000 
known rat genes and 1000 expressed sequence tags 
(ESTs). Five independent gene chips for each group were 
used, resulting in a total of 20 chips analysed. Biotin-
labelled cRNA was fragmented in fragmentation buffer 
(Mes 0.1 M, NaCl 1.0 M, Triton X-100 0.01%, at pH 6.7) and 
herring sperm DNA. Hybridization was performed in an oven at 45°C at 60 rpm for 16 h. Subsequently, arrays 
were washed at 22°C with SSPE-T (NaCl 0.9 M, 
NaH2PO4 60 mM, EDTA 6 mM Triton X-100 0.005%, at pH 7.6) and with 0.1 MES at 45°C for 30 min. The arrays 
were then stained with a streptavidin–phycoerythrin con-
jugate (Molecular Probes, Leiden, The Netherlands) and 
was washed. Supplementary staining with anti-streptavidin anti-
body and streptavidin–phycoerythrin conjugate was used. 
Chips were scanned at a resolution of 3 μm using a confocal 
scanner (model 900154, Affymetrix, Santa Clara, CA). For 
all experimental groups, five chips, each resulting from an 
individual experiment, were of high quality and were used 
for the final analyses.

Analysis of gene expression data
Complex biostatistical analysis of the data has recently been 
described in detail. Briefly, the following data analyses 
were performed.

Normalization and computation of expression values
This procedure was performed using the Robust Multichip 
Average (RMA) method implemented in the module affy 
of the BioConductor open-source bioinformatics software 
(http://www.bioconductor.org/) in the R programming envi-
ronment. Only the perfect match distribution across all arrays 
was used, and the data were log2-transformed.

Clustering analysis
To extract the information content of the data, the gene 
expression matrix was filtered using analysis of variance 
(ANOVA with P-value 0.001), and the data obtained were 
used as input for coupled two-way clustering (CTWC) util-
izing the superparamagnetic clustering algorithm. This 
method identifies submatrices of the total expression matrix, 
whose clustering analysis reveals the partitions of genes and 
samples into stable classes. The transcripts analysed were 
rearranged as ordered by the clustering algorithm, so that 
the transcripts with the most similar expression patterns, as 
measured by the Euclidean distance, were placed adjacent to 
each other. Gene and sample clusters were regarded as stable 
according to specified size and stability index. The following 
parameters were used to optimize the resolution of the 
clustering process: gene cluster size ≥15, sample cluster 
size ≥4, stability threshold of gene and sample clusters 
ΔT≥6 K with one drop-out for samples and three drop-
outs for genes at an increment in T. Iterative scaling and 
merging algorithms were used to preprocess expression data.

Differentially expressed genes and Venn diagrams
A significance analysis of microarrays algorithm (SAM), 
specially designed for the assessment of differential gene 
expression, was used as a software package for the statistical 
analysis of microarray data. SAM provides ranking of genes 
according to their relative importance. A false discovery rate 
between 1% and 3% was used to obtain the lists of differen-
tially expressed genes, from which the Venn diagrams 
were obtained. The software package GenMAPP (http:// 
www.GenMAPP.org) was used to visualize differential 
regulation of important biological pathways between 
A_PreC and A_PostC.

Confirmation of selected transcript levels by quantitative real time reverse transcription polymerase chain reaction (RT-PCR)
RT-PCR was performed for eight selected genes to validate 
microarray data using the primers listed in Table 1. For each
amplification, 20 μl of cDNA were diluted in water (1:10) before being used as a template for the QuantiTect SyberGreen RT-PCR kit (Qiagen, Hilden, Germany). RT-PCR quantification and determination of expression levels were performed on an ABI Prism 7700 Sequence Detector Real-Time PCR machine (Perkin-Elmer, Foster City, CA). Amplification reactions were conducted with an initial step at 90°C for 3 min followed by 20–35 cycles. All PCR reactions were performed in triplicate, and α-tubulin and aminopeptidase were used as reference controls. The predicted size of the PCR products was confirmed by agarose gel electrophoresis.

**Results**

**Anaesthetic pre- and postconditioning afford similar functional and structural protection against ischaemia–reperfusion injury**

A_PreC with isoflurane at 1.5 MAC for 15 min followed by 10 min of washout before 40 min of test ischaemia, or A_PostC with isoflurane at 1.5 MAC administered during the first 15 min of reperfusion improved functional recovery (Table 2) and decreased infarct size (A_PreC: 7[1]% vs ISCH: 36[1]%, P<0.05) when compared with untreated conditions.
hearts to a similar extent (Fig. 2). In contrast with A_PreC, A_PostC exhibited increased coronary flow during the initial phase of reperfusion. However, developed pressure, $\frac{\mathrm{d}p}{\mathrm{d}t}$, end-diastolic pressure and heart rate were improved to a similar extent during the entire reperfusion period in A_PreC and A_PostC when compared with unprotected hearts.

Cardiac gene expression is differentially regulated by anaesthetic pre- and postconditioning: results from SAM algorithm

To characterize genomic responses to A_PreC and A_PostC, gene expression profiles were determined in hearts subjected to A_PreC or A_PostC, and compared with untreated hearts subjected to ischaemia–reperfusion alone. A_PreC and A_PostC profoundly but differentially affected post-ischaemic gene expression (Fig. 3). Compared with unprotected hearts, A_PostC regulated a markedly higher number of transcripts than A_PreC (upregulated genes, 2298 vs 56; downregulated genes, 1328 vs 21). Only 2.1% of significantly upregulated genes (five transcripts, Table 3) and 1.3% of significantly downregulated genes (17 transcripts, Table 4) were jointly regulated in both cardioprotective treatments when compared with unprotected hearts (Fig. 3). The individual lists of ranked genes can be viewed.
in detail in the Supplementary material (available online). Collectively, A_PreC and A_PostC elicit characteristic and, in the main, differential post-ischaemic gene expression profiles in rat myocardium.

Anaesthetic preconditioning, but not anaesthetic postconditioning, induces a gene expression profile similar to non-ischaemic virgin myocardium and prevents activation of deleterious cardiac remodelling. CTWC was used to search for characteristic clusters of genes and samples in the two types of cardioprotection. ANOVA-filtered RMA data (2133 transcripts, \(P=0.001\)) for all four treatment groups (A_PreC \([n=5]\), A_PostC \([n=5]\), ISCH \([n=5]\) and CTL \([n=5]\)) entered the analysis (Fig. 4).

The main cluster G1 broke into eight stable clusters as follows (Fig. 5): G7 and subcluster G2 (559 and 100 genes, respectively, predominantly downregulated in A_PostC and including many transcripts related to inflammation and cell adhesion such as selectin, vascular cell adhesion molecule 1, intercellular adhesion molecule 1, protein kinase R, interleukin 1\(\alpha\), interleukin 1\(\beta\), tumour necrosis factor \(\alpha\); G5 and subcluster G3 (405 and 44 genes, respectively, markedly upregulated in A_PostC and downregulated in ISCH including MAP kinase activated protein kinase 2, transcripts related to electron transport chain, metabolism and many transcripts related to contractile function); subcluster G4 (150 genes exclusively upregulated in A_PostC including genes related to cellular signalling [phospholipase A2, phospholipase C, regulator of G-protein signalling], ion channels [voltage-dependent calcium channel, inward-rectifying ATP-regulated potassium channel] and transcriptional factors such as ring-finger protein 28); G6 (34 genes, exclusively upregulated in ISCH including long interspersed repetitive DNA elements, focal adhesion kinase, genes associated with cardiac remodelling such as fibroblast growth factor, lysyl oxidase, and myosin heavy chain); G8 (49 genes, upregulated in all protocols with test ischaemia including rhoB, early growth response 1 and heat shock proteins 27, 70 and 90); G9 (49 genes, predominantly upregulated in A_PostC and ISCH including transcripts related to extracellular matrix and remodelling such as matrix metalloproteinase 7, tenascin X, elastin, various collagens, drebrin-1 and growth factors).

The main sample cluster S1 broke into two stable clusters as follows: S2 (A_PreC, ISCH, CTL, \(n=15\)) and S3 (A_PostC, \(n=5\)) were identified as stable. Principal component analysis of sample clusters revealed a close genomic relationship between A_PreC and CTL (non-ischaemic healthy myocardium), while A_PostC established a separate post-ischaemic phenotype (Fig. 4c). Collectively, using CTWC cluster analysis, we were able to demonstrate that

### Table 3

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<th>Probe set ID</th>
<th>Gene product</th>
<th>Fold change</th>
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<tbody>
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<td>Transcribed sequences (similar to tensin)</td>
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<td>RNCPOS9R Rat c-fos mRNA</td>
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### Table 4

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<td>Neuritin</td>
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<td>RNREP24R Rat 2.4 kb repeat DNA right terminal region</td>
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<tr>
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<td>Titin</td>
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<td>Rat long interspersed repetitive DNA sequence L1Rn</td>
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Fig 4  Coupled two-way cluster (CTWC) analysis. (A) Global gene expression matrix (heat map) of the 2133 ANOVA-filtered genes. Rows correspond to the genes, and columns correspond to the samples. The color scale denotes the quantification for gene expression based on normalized and centered log₂ RMA values. Blue indicates least and red greatest degree of expression. CTWC provides dendrograms of gene and sample clusters. Each circle of the dendrogram represents a cluster. The gene dendrogram of the eight stable gene clusters is depicted on the left of the matrix. Note that clusters G3 and G4 are subclusters of cluster G5, and G2 is a subcluster of G7, showing common parents in the tree. The sample dendrogram with the two stable sample clusters is shown at the bottom of the matrix. Major splits marked with circles indicate stable clusters. Red digits indicate stability of gene and sample clusters as derived from the algorithm. (B) Distance matrix reordered according to genes. Blue spots on the diagonal axis indicate gene clusters. (C) Principal component analysis of the various treatment groups. Note that A_PreC clusters with healthy virgin myocardium.
differential gene expression patterns characterize A_PreC and A_PostC in the heart. Importantly, A_PreC, but not A_PostC, elicits a post-ischaemic gene expression profile similar to non-ischaemic virgin myocardium, and prevents activation of the myocardial remodelling programme.

**Anaesthetic pre- and postconditioning differentially regulate key biological pathways**

To determine the significance of A_PreC and A_PostC on individual biological pathways, we used GenMAPP, a new tool for viewing and analysing microarray data (http://www/GenMAPP.org). Representative gene ontology maps visualizing metabolic pathways (glycolysis/glucconeogenesis, the tricarboxylic acid cycle and the electron transport chain), calcium handling/cell adhesion/protein biosynthesis and adaptive response to oxidative stress/heat shock response are shown for A_PreC and A_PostC in supplementary figure S1A–D (available online). While A_PostC predominantly downregulated many transcripts in metabolic and stress-related pathways, A_PreC was either ineffective or produced
Discussion

This is the first comparison between pharmacological pre- and postconditioning on a genome-wide scale. Isoflurane, a well-known cardioprotective volatile anaesthetic, was used to elicit protection in isolated perfused rat hearts. Several salient findings emerged from this analysis. First, pre- and postconditioning similarly preserved post-ischaemic function and decreased infarct size in the experimental setting used. Secondly, and in sharp contrast with the first observation, A_PostC as compared with A_PreC induced a largely differential post-ischaemic gene expression profile. A much higher number of transcripts were up- and downregulated in A_PostC when compared with preconditioned or unprotected hearts, and <2% of all differentially expressed transcripts were jointly regulated by pre- and postconditioning. Opposing genomic effects were observed in key metabolic, calcium-handling, and cell–cell interaction pathways. Furthermore, A_PostC suppressed post-ischaemic inflammatory response more effectively (gene cluster G2 of CTWC) and markedly increased expression of many contractile proteins (gene cluster G3 of CTWC). Thirdly, using the unbiased gene discovery approach CTWC, we were able to unravel a gene discovery approach CTWC, we were able to unravel a close molecular relationship between A_PreC and healthy myocardium, but not A_PostC, which itself established a separate characteristic cardiac genotype. Importantly, A_PostC was unable to prevent the activation of the deleterious post-ischaemic remodelling programme. In fact, transcript levels related to myocardial remodelling were even enhanced by postconditioning (gene cluster G9 of CTWC). Collectively, the data presented provide evidence for similar but also opposing genomic responses to pharmacological pre- and postconditioning elicited by volatile anaesthetics in the myocardium. Hence the two protective strategies, albeit affording a high degree of early functional and structural protection, are not interchangeable with respect to their molecular outcome. Inhibition of the remodelling programme by A_PreC, but not A_PostC, could lead to synergistic beneficial effects if the two forms of protection are combined.

Both pre- and postconditioning are endogenous forms of cardioprotection but at opposite ends of ischaemia. The concept of postconditioning has only recently emerged as a promising alternative strategy against ischaemic injury.17 Beneficial effects from staged reperfusion have been known for many years and linked to attenuated biomechanical stress and preservation of microvascular integrity at the time of reperfusion. However, recent studies have unravelled the activation of pro-survival and anti-apoptotic signalling pathways in the myocardium in response to brief repetitive ischaemic episodes (ischaemic postconditioning)18 or pharmacological agents (pharmacological postconditioning)5 applied during early stages of reperfusion, including the phosphatidylinositol-3 kinase protein kinase B (Akt) and the extracellular signal-regulated kinase 1/2 pathways. The same cascades were demonstrated to be operative in cardioprotection by A_PreC,7 and are likely to be involved in protection by A_PostC.19

Based on the comparable degree of functional and structural protection and the known similarities in cellular signalling between A_PreC and A_PostC, we expected to detect identical or similar genomic responses in the myocardium. CTWC clustering was used to identify patterns of genes within the large database. This robust clustering method provides insight that would have been impossible by simply looking at particular gene lists. Unique expression patterns emerged and confirmed the previously reported close genomic relationship between preconditioned and non-ischaemic healthy myocardium.10 However, in sharp contrast to our hypothesis and despite some rather moderate similarities, A_PostC reprogrammed post-ischaemic myocardium differentially compared with A_PreC. Thus, whilst hearts may be protected in the short-term by postconditioning, the results of this study provide evidence that potentially detrimental cardiac remodelling may not be prevented.

Transcripts similarly regulated between anaesthetic pre- and postconditioning (SAM algorithm)

Transcriptional comparison unravelled only 22 out of more than 8000 transcripts expressed in a coordinated manner and shared across A_PreC and A_PostC. Of these, five transcripts were jointly upregulated (Table 3). Tensin, an actin-binding protein, was previously reported to activate the anti-apoptotic c-Jun N-terminal kinase pathway.20 Also, zf36, one of the zinc-finger transcription factors, was overexpressed in both protective strategies. As the zf36 knockout mouse model displays deleterious tumour necrosis factor α overexpression,21 the increased expression of zf36 may result in beneficial anti-inflammatory actions. Interestingly, the hypoxia-inducible factor (HIF)–prolyl hydroxylase,22 the actual oxygen sensor in the tissue, was markedly upregulated in A_PreC and A_PostC. This would imply enhanced post-translational modification and proteasome-mediated degradation of HIF, ultimately preventing the activation of HIF-responsive genes. Conversely, evidence exists to support the existence of HIF stabilization by an Akt pro-survival pathway.23 Many of the 17 jointly downregulated transcripts (Table 4) were related to repetitive DNA elements, particularly the retrotransposable elements LINE1. We have recently reported on this retrotransposon transcriptional burst after ischaemia–reperfusion and its prevention by ischaemic as well as pharmacological preconditioning.10
The current notion that pharmacological postconditioning similarly inhibits activation of these transcripts further reinforces the concept that LINEs may play an important role in ischaemia–reperfusion phenomena. Finally, inhibition of specific cytochrome P-450 isoforms during reperfusion may be protective, which is consistent with recent observations.24

**Opposing regulation of transcripts involved in cardiac remodelling**

Ventricular remodelling is a detrimental adaptive response and starts immediately after myocardial infarction.25 Factors such as hypertrophy, apoptosis and fibrosis participate in this process, which has a high propensity for arrhythmogenesis and sudden cardiac death, and ultimately leads to cardiac failure.26 Consistent with myocyte hypertrophy, A_PostC markedly increased expression of many contractile proteins. Cardiotrophin 1, a hypertrophy-inducing member of the interleukin 6 family which activates pro-survival Akt and extracellular signal-regulated kinase 1/2 and protects against ischaemia–reperfusion injury, was among the highly overexpressed transcripts in A_PostC.27 Immediate early genes (c-fos, EGR-1), as well as fetal genes (brain natriuretic peptide, β-myosin heavy chain) were also overexpressed in A_PostC. Consistent with an effective architectural rearrangement, matrix metalloproteinase 7, responsible for collagen degradation and remodelling of the extracellular matrix,28 together with tenascin, elastin, multiple types of collagen, fibromodulin and insulin-like growth factor II, were upregulated and exclusively clustered in unprotected and postconditioned hearts. Decreased ventricular remodelling29 and improved cardiovascular long-term outcome30 were previously observed in patients with coronary artery disease and an effective preconditioning mechanism. This study now demonstrates for the first time that A_PostC does not inhibit but strongly promotes an early activation of the detrimental postinfarct cardiac remodelling programme.

**Transcriptional differences in the heat shock response, anti-inflammatory actions, and metabolic pathways**

Heat shock proteins serve as molecular chaperones promoting folding and assembly of nascent peptides. Reperfusion of ischaemic rat hearts rapidly activates heat shock factor 1 (HSF1). Surprisingly, HSF1, which increases the expression of many heat shock proteins under cellular stress, was exclusively downregulated in A_PostC. However, this may be due to extracellular signal-regulated kinase 1/2 and c-Jun N-terminal kinase mediated inhibition of HIF1-driven transcriptional activity.31 In addition, double-stranded RNA-dependent protein kinase, which stabilizes the mRNA of heat shock proteins, was highly suppressed.32 Consequently, the expression of many heat shock proteins was suppressed in A_PostC compared with unprotected hearts, while A_PreC selectively enhanced expression of heat shock protein 20.33

Transcripts involved in the inflammatory response were consistently downregulated in all protocols receiving test ischaemia and reperfusion. It is noteworthy that this effect was most pronounced in postconditioned hearts. Although mediators of inflammation are important in ischaemia–reperfusion damage, the observed anti-inflammatory status could reflect a protective counter-regulatory response of the viable myocardium unmasked in the absence of leukocytes.10 In our model, A_PostC, but not A_PreC, markedly reduced the expression of nuclear factor κB, which potentiates transcription of cytokines, chemokines, and cell adhesion molecules. Rat hearts intracoronarily transfected with antisense oligonucleotides containing the nuclear factor κB cis-element prior to coronary ligation showed reduced nuclear factor κB activation during reperfusion and decreased infarct size.34

Reperfusion paradoxically exaggerates ischaemic damage. A burst of free oxygen radicals (ROSs) occurs within the first minutes of reperfusion and significantly contributes to overall damage.35 Reduced ROS formation has been implicated in cardioprotection by postconditioning. However, the source of oxidants has not yet been elucidated. Our analysis now shows, at a gene expression level, that A_PreC and A_PostC differentially regulated important members of the respiratory chain. Complexes I and III, the main sites of oxidant production during ischaemia–reperfusion,36 were particularly downregulated in A_PostC. Interestingly, uncoupling protein 2 (UCP2) was upregulated and clustered in both A_PreC and A_PostC. UCP2 is a proton carrier present in the inner membrane of mitochondria which disperses the mitochondrial proton gradient by bypassing ATP production and oxidative phosphorylation. Overexpression of UCP2 limits the generation of ROS by mildly uncoupling respiration at complexes I and III, thus serving as an antioxidant defence.37 It should be noted that many transcripts of enzymes involved in glycolysis/gluconeogenesis and the tricarboxylic acid cycle including isocitrate dehydrogenase were exclusively downregulated in A_PostC. Decreased mRNA and protein levels of mitochondrial isocitrate dehydrogenase clearly indicate significant mitochondrial oxidative stress.38 This contrasts with multiple transcripts of antioxidant enzymes including catalase, haem oxygenase 1, glutathione peroxidase 1 and superoxide dismutase (isoforms 2 and 3, but not 1), which were exclusively suppressed in A_PostC. Metabolic changes in A_PostC were in many respects similar to previously reported trigger responses of A_PreC with genome-wide downregulation of many metabolic enzymes.910 Downregulation of key metabolic enzymes may be a regulatory response to increased glucose uptake by overexpressed GLUT1 and GLUT3 in A_PostC.39 Finally, slower β-myosin heavy chain actomyosin ATPase activity40 may spare ATP consumption and thus be beneficial for post-ischaemic metabolic and functional recovery.
Opposite regulation in calcium handling and cell–cell interaction

Postconditioning by brief ischaemic episodes possesses strong antiarrhythmic effects against malignant reperfusion-induced arrhythmias. Whether this applies to A_PostC is not yet clear, since anti-arrhythmic effects of A_PreC as opposed to ischaemic preconditioning are also rather controversial. The mechanisms of reperfusion arrhythmias are complex. However, it may be speculated that the observed balanced upregulation of many channels involved in Ca\(^{2+}\) handling may contribute to less heterogeneity of electrical refractoriness and thereby diminish likelihood of re-entrant circuits. Conversely, increased expression of gap junction proteins (connexins 32, 36 and 40, but not 43) was exclusively observed in A_PostC. Altered gap junction coupling during ischaemia and reperfusion may contribute to the development of lethal arrhythmias and propagation of cellular injury. Clearly, additional work is warranted to expand on these findings.

Translational research and clinical implications

Considerable effort has focused on improving perioperative outcome in high-risk surgical patients. Recently, the first placebo-controlled randomized clinical trial demonstrated improved postoperative myocardial function as assessed by decreased NT-proBNP plasma levels after sevoflurane preconditioning in patients undergoing on-pump coronary artery bypass graft (CABG) surgery. Sevoflurane preconditioning also diminished cardiopulmonary bypass associated renal dysfunction in these patients, implying a more global protection of other vital organs. Intriguingly, prospective follow-up of the same patients unravelled an improved 1-year cardiovascular outcome after sevoflurane preconditioning. Although preconditioning has been successful, its use is limited by the inability to predict the onset of ischaemia. In contrast, implementation of protection at the time of reperfusion is predictable and suggests a therapeutic alternative in situations of uncontrolled ischaemic events. Pioneering studies by De Hert and colleagues recently showed that the use of sevoflurane and desflurane throughout the operation in patients undergoing on-pump CABG surgery resulted in improved postoperative cardiac function, decreased release of cTnI and shorter intensive care unit and hospital stays when compared with propofol-based anaesthesia. Administration of sevoflurane only before or after cardiopulmonary bypass resulted in significantly less protection. In contrast, in the experimental setting, the question of whether a combination of pre- and postconditioning is additive remains controversial. However, the results of our study provide molecular evidence to support the use of volatile anaesthetics throughout surgical procedures. Since reiterative ligation and balloon inflation of atheromatous coronary arteries or cross-clamping of a calcified aorta, as required in ischaemic postconditioning protocols, are potentially hazardous, pharmacological pre- and postconditioning by volatile anaesthetics should be preferentially used in the clinical setting.

Study limitations and specific comments

Several factors should be borne in mind in the interpretation of these data. Using the same experimental conditions, we have recently reported a higher number of differentially up- and downregulated genes when A_PreC was directly compared with unprotected hearts. However, the lower number of regulated genes observed in the present experiments is a direct consequence of the altered standardization procedure with the Robust Multichip Average (RMA) method if an additional treatment group, i.e. A_PostC, is included in the normalization and computation of expression values. In fact, an almost perfect match with respect to the number and type of regulated transcripts was obtained between the experiments in the present study and our previous work if A_PostC was excluded from the RMA standardization procedure, reinforcing the strength of the model and data analysis used. This study is an observational study and does not allow ultimate interpretation of the significance of individual transcripts in the protection by A_PreC and A_PostC. However, the results draw attention to possible candidate transcripts, which will permit future hypothesis generation and targeted study of potential protective mechanisms. In addition, changes in mRNA levels may not always correlate with respective protein levels. Also, data from rodent models must always be interpreted with caution when translating to humans. In particular, differences in signalling pathways involved in cytoprotection were previously reported for rat and human myocardium. Therefore future studies should evaluate genomic and proteomic responses to volatile anaesthetics in the myocardium of patients. Finally, confounding ischaemic periods may occur during preparation of the Langendorff hearts, which in addition have only a limited long-term biological stability.

Conclusions

We have used an unbiased gene discovery approach to compare the genomic responses to pharmacological pre- and postconditioning elicited by volatile anaesthetics. A_PreC, but not A_PostC, induces a post-ischaemic gene expression profile similar to virgin myocardium, and prevents early activation of the deleterious cardiac remodelling programme. This study provides for the first time evidence for opposing genomic responses in cardioprotection by pre- and postconditioning, supporting the concept that, despite comparable short-term functional and structural protection, the genomic phenotype and potentially associated long-term outcome may be different.
Supplementary material

Supplementary material is available in British Journal of Anaesthesia online.

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