Editorial

Toward the heart of ischemic preconditioning

Lukas R.C. Dekker *

Department of Clinical and Experimental Cardiology, Academic Medical Center, M-0-54, P.O. Box 22700, 1100 DE Amsterdam, Netherlands

Received 10 September 1997; accepted 11 September 1997

Keywords: Experimental; Heart; Pathophysiology; Preconditioning; Ischemia; Signal transduction

1. Introduction

In 1986 Murry and co-workers introduced the term ‘Ischemic Preconditioning’ [1]. In this classical paper the authors referred to ischemic preconditioning as an adaptation to ischemic stress induced by repetitive short periods of ischemia and reperfusion. The protective effect comprised a reduction of energy consumption and a delay of the onset of lethal cell injury during ischemia resulting in a limitation of infarct size after 40 minutes, but not after three hours of sustained ischemia [1]. The fascination for this intrinsic protective property of the myocardium has led to a still expanding gamut of research, which was comprehensively reviewed by Yellon and co-workers in this issue of Cardiovascular Research [2].

Three aspects can be distinguished in the process of ischemic preconditioning. The initial trigger (1), that is included in the short periods of ischemia and reperfusion, activates signalling pathways (2), which in their turn act upon an end-effector inducing the delay of lethal ischemic damage during sustained ischemia (3). All three facets have been extensively studied, although the vast majority of the investigations focuses on the signalling pathways. In spite of this, the central mechanism, which results in the delay of necrosis, remains unclear. Therefore, it can be questioned whether the apparently centrifugal expansion of triggers, mediators and salutary endpoints of ischemic preconditioning [2,3] will at any time lead us to clarify a central effector mechanism. This paper aims at expounding the concept of the central mechanism and critically reviews the still increasing number of proposed signalling pathways and salutary endpoints of preconditioning.

2. The trigger of ischemic preconditioning

Obviously, the factor initiating protection is operative during the short periods of ischemia or reperfusion of ischemic preconditioning. However, this factor remains obscure. The majority of evidence suggests that it is the ischemia and not the reperfusion that starts the preconditioning cascade. Schulz and co-workers showed that a 10-min period of no-flow ischemia provides protection against subsequent low-flow ischemia without intermittent reperfusion [4]. Since adenosine is produced by degradation of ATP during the short periods of ischemia and activates a protective cascade when administered, it has been proposed as a trigger for ischemic preconditioning [5]. Also, bradykinin may act as a trigger [6]. However, several observations mitigate the importance of adenosine and bradykinin in triggering preconditioning. Adenosine and bradykinin are not important for preconditioning in rats [7,8]. Also, hypoxic perfusion, preventing extracellular accumulation of adenosine is as effective as ischemia in inducing preconditioning [9]. Moreover, antagonizing the bradykinin B2 receptor does not prevent cardioprotection after 4 cycles of short periods of ischemia and reperfusion in rabbit hearts [6].

An alternative candidate for initiating ischemic preconditioning is a preischemic mild increase of the intracellular Ca2+ concentration ([Ca2+]i). Short periods of ischemia and reperfusion induce a loss of Ca2+ from the sarcoplasmic reticulum (SR) and an accumulation of Ca2+ in the cytosol [10–12]. Recently Miyawaki and co-workers inferred that a small increase of [Ca2+]i acts as a mediator of preconditioning via activation of protein kinase C (PKC) [13,14].

* Corresponding author.

0008-6363/98/$19.00 © 1998 Elsevier Science B.V. All rights reserved.
PII S0008-6363(97)00241-1
If the intensity of the preconditioning stimulus exceeds a certain threshold by extending or frequently repeating the period of ischemia, the protective effect is lost [15]. Other ‘subthreshold’ noxious interventions, including hypoxia [9], stretch [16] and a mild increase of \( [\text{Ca}^{2+}] \) [13], also initiate a protective effect and may share a common stimulus with ischemic preconditioning. However, such comparison does not stand up for preconditioning the myocardium with drugs. In pharmacological preconditioning the period of drug treatment is in principle separated from the start of ischemia by a drug free episode. However, it has never been clarified whether the drugs are completely washed out prior to ischemia [17]. In any case, a drug-free interval in pharmacological preconditioning is not a prerequisite for protection, in contrast to a period of reperfusion in ischemic preconditioning. Therefore, pharmacological preconditioning might not be helpful to elucidate the trigger and initial steps of the mechanism underlying ischemic preconditioning. Instead, detailed biochemical analysis of myocardium during periods of ischemia and reperfusion may provide the key to the understanding of ischemic preconditioning [18].

3. The endpoint of ischemic preconditioning

The classical endpoint of preconditioning is the delay of lethal injury reducing infarct size during a temporary coronary occlusion [1]. In line with these initial observations various studies have demonstrated that specific manifestations of the onset of ischemic myocardial necrosis are postponed by preconditioning. The terminal rise in \( [\text{Ca}^{2+}] \) is delayed by ischemic preconditioning [18–20]. Also, the development of ischemia-induced ultrastructural damage is slowed by preconditioning [21]. The delay of the onset of ischemia-induced electrical uncoupling is another manifestation of protection against ischemic damage by preconditioning [20,22,23]. However, reports in the literature about the effect of preconditioning on the start of contracture are conflicting, probably depending on differences in species and experimental models. Studies in both rat and rabbit hearts [20,24,25] show that preconditioning postpones the start of contracture during sustained ischemia compared to control hearts. Opposite results showing that preconditioning advances ischemia-induced contracture during sustained ischemia have been obtained exclusively in rats [26,27]. This discrepancy is surprising, since preconditioning delays the development of necrosis, while contracture is strongly indicative of irreversible ischemic damage and necrosis [28].

Several salutary endpoints have been described in addition to the delay of lethal ischemic damage and infarct size reduction. However, compelling evidence is currently lacking to conclude that these endpoints comprise different signalling pathways and different mechanisms. Therefore, there is no ground to renounce that the delay of ischemic damage is the common basis of all parameters of cardioprotection.

3.1. Infarct size reduction

Infarct size reduction is the prevailing measure of cardioprotection. The transmural progression of the infarct from endocardium to epicardium during acute myocardial ischemia [29] is not interrupted, yet merely retarded [21]. Therefore, infarct size reduction is lost when sustained ischemia is extended to three hours, as already noted in the first paper on preconditioning [1]. In short, ‘infarct size reduction’ after a temporary coronary occlusion is a consequence of the delay of ischemic cell death.

3.2. Antiarrhythmic effects

A preconditioning-induced reduction of the incidence and severity of arrhythmias during ischemia and reperfusion has been described primarily in rats [30–32], but also in dogs and humans [33,34]. Other studies in pigs and dogs have demonstrated an absent or stimulating effect of preconditioning on the occurrence of arrhythmias [1,35].

It is premature to conclude that the reduced incidence of arrhythmias by preconditioning is due to an antiarrhythmic effect per se and not to the ‘classical’ delay of ischemic cell death. The rat is not a representative model to study ischemia-induced arrhythmias. The action potential characteristics of rat ventricle, and their changes during ischemia, are different compared to larger mammals. Furthermore, the small size of rat hearts probably precludes the division of ischemia-induced arrhythmias into the phase 1a and 1b arrhythmias, which normally occur in larger hearts and have been shown to depend on different arrhythmogenic mechanisms [36,37].

As infarct size by itself is an important determinant of the incidence and severity of ventricular arrhythmias [38,39], the reduction of the incidence of arrhythmias in preconditioned myocardium is, at least partially, caused by the limitation of infarct size during early ischemia [40]. Therefore, equal infarct sizes between control and preconditioned groups are mandatory to decide whether preconditioning has a true antiarrhythmic effect.

The electrophysiological derangements underlying phase 1a and 1b arrhythmias during ischemia, such as ST-segment elevation and electrical cellular uncoupling, are attenuated and postponed after preconditioning, respectively [20,22,41]. Accordingly, Cinca and co-workers recently showed that ischemic preconditioning postpones electrical uncoupling as well as the 1b phase of arrhythmias during sustained ischemia in pigs [23]. The slowing of the ischemic changes and the reduction of the size of the infarcted area underlie the reduced propensity of preconditioned myocardium to arrhythmias during ischemia and reperfusion.
3.3. Reduction of contractile dysfunction

It has been demonstrated in many species that ischemic preconditioning attenuates postischemic myocardial contractile dysfunction [26,27,42]. This beneficial effect is not caused by a reduction of stunning, because preconditioning does not protect against stunning [43,44]. Improvement of postischemic function by preconditioning is secondary to the delay of ischemic myocardial damage, because parameters of the degree of necrosis, such as infarct size and enzyme leakage, correlate with the enhancement of functional recovery [42,43].

3.4. Clinical applications

Although ischemic preconditioning might occur under certain clinical conditions (for review see Verdouw and co-workers [45]), this does not mean that it constitutes a promising tool for daily clinical practice [46]. Recent experimental investigations have demonstrated that in myocardium affected by certain preexisting pathological conditions ischemic preconditioning does not have its desired effects. In aging rat hearts the beneficial effects of preconditioning are lost or reversed [47,48]. Furthermore, we have recently shown that in hearts from rabbits suffering from severe heart failure preconditioning may paradoxically advance markers of the onset of ischemic damage [49].

4. Signalling pathways of ischemic preconditioning

In contrast to the scant knowledge about the mechanisms underlying the initiating event and the endpoint of ischemic preconditioning, the potential signalling pathways have been, and continue to be, extensively studied. Unfortunately, the interrelations between the stimulus, the intracellular transduction pathways and the final effector mechanism remain unknown at present.

4.1. \(A_1\) adenosine receptor

In addition to its role as a trigger, it has also been suggested that adenosine receptor binding acts as a mediator of preconditioning [5]. Protective effects after activation of the \(A_1\) adenosine receptor have been described in many species [50,51]. The inhibitory G (G\(_i\)) protein and protein kinase C (PKC) have been proposed as mediators of adenosine-mediated cardioprotection [52,53]. However, as already mentioned above, adenosine may not be the key factor of preconditioning. The absent role of adenosine in rats and the persistent protection by ischemic preconditioning in the absence of extracellular accumulation of adenosine preclude an important role for adenosine [7,9,54].

4.2. ATP-sensitive K\(^+\) channels

In normal myocardium ATP-sensitive K\(^+\) channels (\(K_{ATP}\)) are closed at physiological intracellular concentrations of ATP [55]. From studies using openers and blockers of these channels it has been hypothesized that activation of \(K_{ATP}\) channels plays a role in ischemic preconditioning [22,56]. Action potential shortening after opening of \(K_{ATP}\) channels was assumed to reduce Ca\(^{2+}\) influx via the voltage-sensitive Ca\(^{2+}\) channels protecting the myocardium during ischemia [57]. However, it has now become clear that low dosages of the \(K_{ATP}\) channel opener bimakalim has potent cardioprotective effects in the absence of action potential shortening precluding the above hypothesis [58]. Interestingly, a recent study by Armstrong and co-workers has indicated that \(K_{ATP}\) channel opening may act as an initiator of preconditioning via a PKC-dependent pathway [59].

4.3. Protein kinase C

It has been proposed that activation of G protein coupled receptors, including \(\alpha_{1B}\) adrenergic, \(A_1\) adenosine, muscarinic and endothelin receptors, plays an important role in ischemic preconditioning by inducing a translocation of PKC to the membrane [2,60–64]. However, downstream of this point in the supposed PKC-mediated pathway of preconditioning information and even hypotheses are lacking at present.

As with the other mediators, the role of the non-selective second messenger PKC in preconditioning has become subject to considerable controversy [65]. Several recent publications, in which PKC inhibition did not prevent cardioprotection [66,67], or even induced an additional protective effect [68], seriously dispute the role of PKC in preconditioning. A confounding factor in examining the effects of PKC is the existence of at least 6 isoforms with their own characteristics in cardiac tissue [69]. To date, the relative roles of these isoforms in the protective effect of preconditioning, if any, are unclear.

4.4. Other possible mechanisms

Other mechanisms, that will not be discussed in further detail (for review see [2,3]) have been proposed. Arachidonic acid [19], stretch [16], heat stress proteins [70] and mitogen-activated protein kinases [71] (MAP kinases) have all been associated with preconditioning. Attempts have been made to unify some of the aspects of the apparently diverging field of triggers and mediators in preconditioning. For instance, one possible effector mechanism of the \(A_1\) adenosine receptors is the activation of ATP-sensitive K\(^+\) channels via a G\(_i\) protein mediated process [52,72]. Alternatively, a synergistic modulation of \(K_{ATP}\) channels by PKC and adenosine has been described.
5. The mechanism of the delay of ischemic damage

The common feature characterizing the protected state of preconditioned myocardium is a reduction of energy demand causing a delay of necrosis during sustained ischemia [21]. Although preconditioning reduces mitochondrial ATP by about 30%, the rate of decline during subsequent sustained ischemia is decreased and only after 15 minutes of ischemia ATP content in preconditioned and control hearts is similar [18,21,74]. As glycogen utilization is depressed after preconditioning, the slower rate of high energy phosphate depletion during sustained ischemia can only be a consequence of a reduced energy consumption during ischemia in preconditioned myocardium compared to ischemia in control myocardium [18,21,75]. Murry and co-workers proposed that this initial reduced rate of anaerobic glycolysis attenuates the accumulation of glycolytic products, including lactate, and that this could be responsible for delaying cell death [21]. In support of this idea are studies showing that preconditioning slows the development of acidosis during sustained ischemia [18,26,75].

A pivotal hiatus in the understanding of preconditioning is that the fundamental central mechanism underlying the reduction of energy consumption and the delay of necrosis during sustained ischemia remains unclear. Attenuation of contractile function by the brief periods of ischemia and reperfusion (‘stunning’) does not underlie decreased energy consumption in preconditioning. Murry and co-workers have shown that the protective effect of a 15-min period of preconditioning ischemia is lost after 120 min, whereas stunning is still present indicating that the time windows of protection and stunning are different [76]. Furthermore, elucidating stunning, via hypoxic preconditioning or administration of dobutamine, does not prevent protection [77,78]. Another important pathway consuming energy during early ischemia is the reversal of the mitochondrial ATPase. However, this also does not play a role in the energy sparing effect of ischemic preconditioning, since ATP hydrolysis by the mitochondrial ATPase during ischemia is inhibited within the first 90 s of ischemia in control and preconditioned cardiac tissue [79].

In pharmacological preconditioning the drug-free interval is not essential for the protective effect, in contrast to the period of reperfusion in ischemic preconditioning (see above). Therefore, one should ponder on the question whether the mechanisms underlying the protective effects of pharmacological preconditioning are essentially different from the long-established increase of ischemic tolerance by preischemic treatment with agents like propranolol or verapamil [80,81]. Moreover, these considerations also indicate that it is not self-evident that mechanisms underlying pharmacological preconditioning can be extrapolated to ischemic preconditioning.

It is tempting to speculate on a novel common mechanism of ischemic preconditioning. For example, [Ca^{2+}], may play an important central role. Various studies have demonstrated that short periods of ischemia and reperfusion temporarily induce a mild increase of [Ca^{2+}], by decreasing SR Ca^{2+} uptake [10,12] or increasing SR Ca^{2+} efflux [10,11]. It can therefore be postulated that a mild increase of [Ca^{2+}], initiates cardioprotection during ischemic preconditioning by activating protein kinase C (PKC) [13,14]. Furthermore, this mild increase of [Ca^{2+}], may also represent the effector mechanism of cardioprotection. Steenbergen and co-workers have previously suggested that the degree of SR Ca^{2+} loading is an important determinant of the rate and extent of irreversible damage during ischemia, since the SR Ca^{2+}-ATPase consumes a substantial portion of total cellular energy under ischemic conditions [82–84]. Therefore, the reduction of the Ca^{2+} gradient across the SR membrane induced by the short periods of ischemia and reperfusion is thermodynamically favorable and may constitute a cardioprotective mechanism. This has been confirmed by various studies showing that drugs that deplete the SR, such as caffeine or low doses of ryanodine protect the myocardium during subsequent ischemia or metabolic inhibition [85,86]. In addition, one of the effects of activated PKC is inhibition of Ca^{2+}, accumulation in the SR [87,88]. Therefore, Ca^{2+}-dependent PKC activation and PKC-mediated transition of Ca^{2+}, from the SR into the cytosol may constitute a positive feedback loop resulting in a thermodynamically favorable and protected status of the myocardium.

In conclusion, ischemic preconditioning definitely is a powerful means to protect the ischemic myocardium. However, current research on the different aspects of preconditioning seems to diverge more and more. Although this provides numerous interesting new data, the central mechanism of ischemic preconditioning remains obscure. Furthermore, pharmacological preconditioning is not necessarily an analog of ischemic preconditioning. Therefore, this paper is meant as an impetus to focus on the basic and essential factors of cell death and the reduction of energy consumption during ischemia.
References

[1] Murry CE, Jennings RB, Reimer KA. Preconditioning with isch‐


[15] Ilidromitis EK, Kremastinos DT, Katrisis DG, Papadopoulos CC, Hearse DJ. Multiple cycles of preconditioning cause loss of protec‐


[19] Murphy E, Glasgow W, Fralix TA, Steenbergen C. Role of lipopy‐


[21] Murry CE, Richard VJ, Reimer KA, Jennings RB. Ischemic precon‐


[32] Lawson CS, Colta DJ, Hearse DJ. ‘Dose’-dependency and tempo‐

emia-induced ventricular arrhythmias in variant angina. Circula‐


[42] Cohen MV, Liu GS, Downey JM. Preconditioning causes improved


[60] Liu Y, Ytrehus K, Downey JM. Evidence that translocation of protein kinase C is a key event during ischemic preconditioning of rabbit myocardium. J Mol Cell Cardiol 1994;26:661–668.


