Editorial

Dysfunctional ischemic preconditioning mechanisms in aging

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Received 1 November 2000; accepted 1 November 2000

See article by Tani et al. [7] (pages 56–68) in this issue.

1. Introduction

With increased age, the adult heart undergoes numerous anatomical, ultrastructural and biochemical changes which remodel cellular structure, function and the intracellular adaptive response to pathological stress [1–6]. Compared to young adult myocardium, the senescent myocardium has a reduced capacity to tolerate post-ischemic perturbations in intracellular metabolism and ion homeostasis [1–6]. In this issue of Cardiovascular Research, Tani et al. [7] report that there is an age-linked functional decline in protein kinase C (PKC) activation, a crucial element in the cardioprotective signaling pathways triggered by ischemic preconditioning. Lethal ischemia-reperfusion injury can be attenuated by the heart’s intrinsic capacity to be ‘pre-conditioned’ by a variety of metabolic and pharmacological stimuli, including sub-lethal episodes of ischemia itself [8]. This discovery has generated intense investigation of the preconditioning phenomena which, despite considerable variability, has been validated in a large number of experimental models and species. As a platform for considering this report, let us consider some key aspects of the exciting new developments in the study of the mechanisms underlying preconditioning.

2. Mediators of preconditioning

Although ischemic preconditioning signaling pathways are incompletely defined, there is considerable agreement that the initial steps involve the stimulation of G-protein-coupled receptors such as adenosine A1, A3, α1-adrenergic, bradykinin, endothelin, angiotensin, δ-opioid peptide and muscarinic receptors [9–15]. Activation of specific G-proteins (depending on the respective G-protein-coupled receptor type) causes generation of inositol (1,4,5) tris-phosphate (IP3) and diacylglycerol, the endogenous substrate for PKC activation via the classical phospholipase activation pathway [16]. IP3 has been shown to activate IP3 receptors on the sarcoplasmic reticulum of cardiac myocytes [17]. Although it is unclear whether IP3 has a role in ischemic preconditioning, recently it has been reported that preconditioning evokes a biphasic fluctuation in myocardial IP3, with an increase in IP3 during ischemic preconditioning and a decrease during sustained ischemia [18]. Notably, treatment with an IP3 agonist followed by an IP3 antagonist has been reported to mimic the protective effects of preconditioning [18].

Antagonists of PKC activation may block ischemic or receptor-induced preconditioning [19–21], whereas functional protection against global ischemia/reperfusion injury can be induced by diacylglycerol [18,22]. Although controversial, up to 11 PKC isoforms have been found in the heart [16,23–28], each with their own particular condition for activation, intracellular site and substrate specificity. It has been proposed that activation and translocation of PKC isoforms includes complex conformational changes that ultimately expose residues to permit binding to specific receptors for activated kinases (RACKs) and these in turn localize activated PKC subunits to specific subcellular targets [16,25,26]. Activation and translocation of specific PKC subunits to the sarcolemma, myofilaments, cytoskeleton and organelle membranes may result in modification of ion homeostasis, pH and contractile function [23–28]. PKC-dependent ischemic preconditioning pathways are further complicated by a number of exciting recent findings that suggest the additional involvement of p42/44-mitogen activated protein kinase (MAPK) and p38-MAPK signaling [29,30].

Since the discovery of KATP channels in mitochondria [31], there has been strong focus on finding that opening of...
$K_{\text{ATP}}$ channels are central to mediating ischemic or pharmacological preconditioning [32–34]. The regulation of mitochondrial $K_{\text{ATP}}$ channel opening is dependent upon activation by cytosolic guanine nucleotides (GTP, GDP) and ATP [35]. Long chain acyl-CoA esters in the presence of Mg$^{2+}$ inhibit the mitochondrial $K_{\text{ATP}}$ channel opening induced by diazoxide, but this inhibition can be reversed by cytosolic guanine nucleotides [35]. It has been shown that specific blockade of mitochondrial $K_{\text{ATP}}$ channels by 5-hydroxydecanoic acid prevents the cardioprotective action of ischemic or pharmacological preconditioning [32]. $K_{\text{ATP}}$ channel opening is proposed to promote dissipation of proton motive force-generated membrane potential, inhibition of the mitochondrial calcium influx via the uniporter, and inhibition of mitochondrial ATPase, thus protecting against ischemia-induced calcium overload and conserving ATP [32]. It has also been proposed that the opening of mitochondrial $K_{\text{ATP}}$ channel may regulate mitochondrial volume, a key factor that maintains the structure and function of the inner membrane which is crucial to the regulation of the mitochondrial electron transport system energy production and transfer [33]. The specific mechanism of cardioprotection exerted by the opening of mitochondrial $K_{\text{ATP}}$ channels, however, remains highly controversial and is yet to be defined.

Notably, it has been reported that ischemic preconditioning can induce activated PKC-δ to be translocated to the mitochondria of rat cardiac myocytes [36]. A recent study finds that PKC-ε and not PKC-δ plays a principal role in mediating ischemic preconditioning in rabbit cardiac myocytes [37]. It remains to be established whether during ischemic preconditioning PKC activates mitochondrial $K_{\text{ATP}}$ channels directly or does so indirectly via tyrosine kinases downstream of PKC [38].

While some have hailed mitochondrial $K_{\text{ATP}}$ channels as the ‘elusive’ final effectors of preconditioning, it is now apparent that additional factors are involved in preconditioning. Reactive oxygen species (ROS) that are produced during brief myocardial reperfusion or reoxygenation have been proposed to trigger cardioprotective preconditioning [15,39]. The formation of ROS during initiation of preconditioning is dependent on mitochondrial $K_{\text{ATP}}$ channel opening [15]. Notably, when myxothiazol specifically inhibits superoxide generated by mitochondrial complex III (at cytochrome $b-\text{C}_{1}$), the effects of preconditioning can be abolished [15,39]. Blockade of the mitochondrial anion channel also prevents mitochondrial release of ROS and preconditioning [39]. In intact hearts, direct uncoupling of the electron transport chain with dinitrophenol, or direct inhibition with cyclosporin A, have been shown to invoke preconditioning-like effects [40]. The recent discovery of ‘ROS-induced ROS release’ indicates that ROS play a crucial signaling role in the regulation of the mitochondrial membrane permeability transition (MPT) pore complex and mitochondrial homeostasis per se [41]. In light of the preceding and a relationship between MPT-induced pore opening and increased likelihood for pro-apoptotic events [42], an important finding is that the attenuation of apoptosis contributes to the diminished ischemic injury observed after ischemic preconditioning [43–45].

3. Effect of aging on ischemic preconditioning

In recent years, it has been recognized that the cardioprotective effects of ischemic preconditioning may be less potent or attenuated by advanced age. In studies with isovolumic, isolated rat heart preparations, pharmacological, hypoxic and ischemic preconditioning strategies failed to prevent post-ischemic contractile dysfunction in isolated middle aged and senescent rat hearts [46–49]. The reduction in post-ischemic release of creatine kinase in coronary effluent, the post-ischemic increase in concentration of myocardial high energy phosphates, and protection against ischemic-induced myocardial necrosis that are normally associated with preconditioning were no longer evident in middle aged and senescent rat hearts [47,49].

The report in this issue of Cardiovascular Research by Tani et al. [7], is the first to examine the effect of age on PKC activation and $K_{\text{ATP}}$ channel activity during ischemic preconditioning of isolated rat hearts. Although cardioprotection due to ischemic preconditioning or 1,2-dioctanoyl-sn-glycerol (DOG) was attenuated in middle-aged rat hearts, diazoxide afforded similar protection (via opening of $K_{\text{ATP}}$ channels) against post-ischemic injury and contractile dysfunction in young adult and middle-aged hearts.

PKC isoform translocation was studied in young and middle aged rats following activation by ischemic preconditioning or 1,2-dioctanoyl-sn-glycerol (DOG) by using PKC isoform-specific antibody labeling of sections. In young hearts, ischemic preconditioning translocated PKC-α from the cytosol to the sarcosomal membranes, PKC-δ from the sarcosomal to the perinuclear and other membrane regions. PKC-η was translocated from the sarcosomal membrane to the cytosol and PKC-ε remained distributed in the cytosol. In contrast, middle aged hearts had PKC-α, ε, and η located mainly in the cytosol while PKC-δ was present in membrane regions. After ischemic preconditioning of middle aged hearts negligible translocation of PKC-isoforms was evident. DOG stimulated translocation of PKC-α, ε, and δ to perinuclear or membrane regions regardless of age. DOG or ischemic preconditioning had no apparent effect on total PKC activity in any age group. When hearts were examined for PKC isoforms by immunoblot analysis method-dependent differences were observed. For both age groups, PKC isoforms were initially predominantly cytosolic. After preconditioning PKC isoforms were located mainly in membrane fractions from young hearts whereas no major
changes in distribution were apparent in middle-aged hearts.

4. Conclusion

Preconditioning signaling pathways that commence at a number of different endogenous transmitter-activated G-protein-coupled receptors appear to converge at PKC. The present study by Tani et al. [7], is the first to report that PKC activation and translocation in ischemic preconditioning are perturbed early in the process of senescence (middle-age). Although downstream of PKC, $K_{\text{ATP}}$ channel-mediated protection against ischemia and reperfusion remained intact in these middle-aged rat hearts. However, it remains to be tested whether, with more advanced age (or different models of aging), preconditioning signaling is more extensively altered. During senescence cell membrane remodeling occurs which can alter intracellular membrane function [1,4]. This remodeling may have an impact on structural and functional modification of membrane proteins and lipids, redox and antioxidant capacity, ion homeostasis and energy metabolism, thus potentially contributing to diminished capacity for preconditioning signaling. However, the elements that contribute to preconditioning, but are diminished in senescence, may possess an intrinsic plasticity for repair or renewal. A recent study has shown that isolated perfused hearts from exercise-trained senescent rats were protected against post-ischemic contractile dysfunction by ischemic preconditioning [50]. In contrast, ischemic preconditioning did not prevent post-ischemic contractile dysfunction in isolated hearts from sedentary senescent rats.

The study by Tani et al. [7] opens up the way for more detailed studies to specifically examine whether age-related dysfunction in ischemic preconditioning is due to changes in: (1) PKC proteins; (2) PKC activation mechanisms; (3) age-associated changes in the specific sites targeted by each PKC isomer. Moreover, the direct mitochondrial relationship between the recruitment of a specific PKC isomer(s) and $K_{\text{ATP}}$ channel opening during ischemic preconditioning remains to be established. The present work is restricted by a limitation in our current understanding of the physiological significance of specific PKC isomer recruitment to target sites. In particular, detail regarding the specific functional relationship between PKC isomer activation, $K_{\text{ATP}}$ channel activity, the electron transport system and MPT pore in the mitochondria awaits definition. Whether the present findings are related to the species or experimental model employed is unclear and requires intense scrutiny. Further study of ischemic preconditioning signaling, particularly in well defined models of aging, will assist understanding of the molecular basis of ischemia and reperfusion injury and the augmented vulnerability to pathogenesis evident in the senescent heart.

References

[14] Schultz JE, Hsu AK, Gross GJ. Ischemic preconditioning in the intact rat heart is mediated by $\delta_1$ but not $\mu$- or $\kappa$-opioid receptors. Circulation 1998;97:1282–1289.


Bauer B, Sinikov BZ, Klöner RA, Przyklenk K. Preconditioning-induced cardioprotection and release of the second messenger inositol (1,4,5)-trisphosphate are both abolished by neomycin in rabbit heart. Basic Res Cardiol 1999;94:31±40.


Garlid K. Opening mitochondrial \( K_{ATP} \) channel in the heart — what happens and what does not happen. Bas Res Cardiol 2000;95:275±279.
