Basic Res Cardiol 101: 354–356 (2006)
DOI 10.1007/s00395-006-0589-0

REPORT

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No loss of cardioprotection by postconditioning in connexin 43-deficient mice

Received: 9 February 2006
Returned for 1st revision: 17 February 2006
1st revision received: 20 February 2006
Accepted: 21 February 2006
Published online: 27 March 2006

Dr. G. Valen, Oslo, Norway, served as guest editor for the manuscript and was responsible for all editorial decisions, including the selection of reviewers. The policy applies to all manuscripts with authors from the editor's institution.

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Abstract In situ hearts and isolated cardiomyocytes from heterozygous connexin 43-deficient ($Cx43^{+/-}$) mice cannot be protected by ischemic preconditioning or diazoxide. We have now addressed the role of connexin 43 in ischemic postconditioning (PC). Wild type (WT) and $Cx43^{+/-}$ mice were subjected to 30 min coronary occlusion and 120 min reperfusion, with and without a PC protocol of three cycles of 10 s coronary occlusion/10 s reperfusion. Infarct size (TTC staining) was reduced by PC from 54±5 to 37±3% of area at risk in WT. Likewise, infarct size was reduced by PC from 53±4 to 34±3% of area at risk in $Cx43^{+/-}$. We conclude that connexin 43 is no prerequisite for PC's protection. To this end, the signal transduction of ischemic preconditioning and postconditioning differs.

Key words Myocardial ischemia – reperfusion – signal transduction – ischemic postconditioning – myocardial infarction

Introduction

Connexin 43 is the major isoform expressed in mammalian, including human, ventricular myocardium. Connexin 43 is located in the sarcolemma, and essential for gap junction formation. The role of connexin in myocardial ischemia/reperfusion and ischemic preconditioning has until recently been attributed to its function in gap junctions and to the spread of injury or protection through gap junctions [12]. In situ hearts from heterozygous connexin 43-deficient ($Cx43^{+/-}$) mice cannot be preconditioned by preceding ischemia/reperfusion (I/R) episodes [13, 14] or diazoxide [6]. Such lack of protection by ischemic preconditioning [9] or diazoxide [6] is also observed in isolated cardiomyocytes from $Cx43^{+/-}$ mice, thus excluding gap junctions as the site where connexin 43 exerts its role in the observed cardioprotection. Instead, we have recently demonstrated the presence of connexin 43 in cardiomyocyte mitochondria and an increased mitochondrial connexin 43 localization with ischemic preconditioning [1] as well as a specific functional defect of cardiomyocytes from $Cx43^{+/-}$ mice to generate reactive oxygen species in response to diazoxide [6].

Ischemic postconditioning (PC) is the reduction of infarct size by several cycles of I/R that follow a sustained ischemic insult, rather than precede it as in ischemic preconditioning [8, 16, 18]. PC shares sig-

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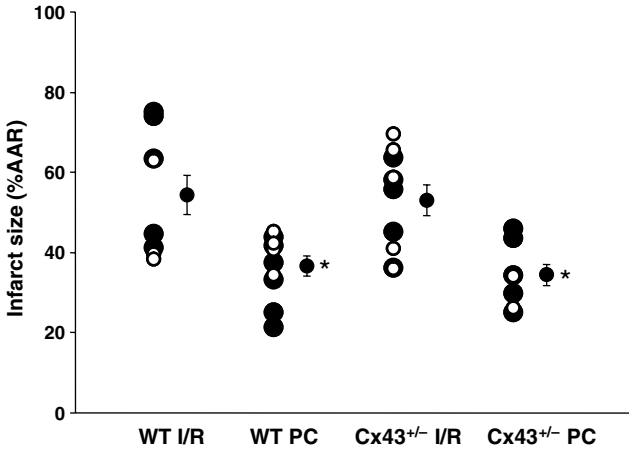

Fig. 1 Infarct size as a percent of the area at risk in wild type (WT) and connexin 43-deficient ($Cx43^{+/-}$) mice with 30 min ischemia/120 min reperfusion (I/R) and with additional PC by three cycles of 10 s ischemia/10 s reperfusion (PC). Results from individual animals (male: closed symbols; female: open symbols) and mean values±SEM. *$P<0.05$ vs. I/R

naling elements with preconditioning [7, 16] and the phenomena possibly converge in the activation of RISK kinases [5] during early reperfusion [3]. PC is also operative in man [15].

We have now addressed the role of connexin 43 in infarct size reduction by ischemic PC.

Methods

Myocardial infarction was induced in $Cx43^{+/-}$ mice and their wild-type littermates (WT), 10–15 weeks of age, as previously described [13, 14]. The left anterior descending coronary artery was occluded for 30 min and reperfused for 120 min. PC was induced by three cycles of 10 s occlusion/10 s reperfusion each, starting at 10 s reperfusion following the sustained ischemia. A total of 10 WT and 10 $Cx43^{+/-}$ were subjected to I/R only, 10 WT and 8 $Cx43^{+/-}$ were subjected to ischemic PC before I/R.

Area at risk was determined by Evans blue staining and infarct size was determined, after removal of the heart, by TTC [14]. Area at risk and infarct size were compared by 2-way ANOVA, followed by Fisher's least-significance tests. $P<0.05$ was considered significant.

Results

Area at risk and infarct size following I/R were not different between WT and $Cx43^{+/-}$. PC reduced infarct size in both WT and $Cx43^{+/-}$ mice to a comparable degree (Fig. 1).

Discussion

The lack of difference in infarct size per se between WT and $Cx43^{+/-}$ mice confirms our prior findings [13, 14]. However, in contrast to ischemic preconditioning or diazoxide [6, 13], connexin 43 appears not to be important for the cardioprotection obtained by ischemic PC. Therefore, the possibly mitochondrial [1, 6] signaling step that involves connexin 43 is more proximal in the triggering or mediating rather than the more distal executing signal transduction, which involves the activation of RISK kinases during early reperfusion [3, 5].

Since connexin 43 appears to be a prerequisite for mitochondria to generate a specific free oxygen radical signal [6], it is at first sight not surprising that connexin 43 is not important for PC during early reperfusion when there is an increased formation and release of free oxygen radicals [2]. However, the issue of mitochondria and free radicals in PC is more complex: mitochondrial K_{ATP}-channels are not only important for ischemic preconditioning [11, 17], but also for ischemic PC [10, 16]. Connexin 43, however, is a prerequisite only for ischemic preconditioning and the purported mitochondrial K_{ATP} opener diazoxide to induce free radical formation and protection [6]. Ischemic PC appears also to rely on a certain free radical signal and is abolished by the free radical scavenger *N*-acetyl-cysteine [4, 10]. Apparently, the role of mitochondria and free radicals is very specific in both the preconditioning and PC phenomena. Clearly and in difference to Downey's and Cohen's notion of a common signal transduction pattern for ischemic preconditioning and PC [3], the involvement of connexin 43 distinguishes the two phenomena.

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