

## Reduced Susceptibility to Ischemia-Induced Arrhythmias in the Preconditioned Rat Heart Is Independent of PI3-Kinase/Akt

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### Summary

We examined the involvement of phosphatidylinositol 3-kinase (PI3K) and its effector protein kinase B (Akt) in cardioprotective effects of ischemic preconditioning (PC) with particular regards to its role in the protection against ischemia-induced arrhythmias in isolated perfused rat heart. PI3K/Akt inhibitor wortmannin (100 nM) was administered 15 min prior to 30-min regional (left anterior descending coronary artery occlusion) ischemia for the study of ischemic arrhythmias in the hearts perfused at constant coronary flow or prior to 30-min global ischemia followed by 2-h reperfusion for the infarct size (IS) determination (tetrazolium staining) in the hearts perfused at constant pressure. PC procedure (one cycle of ischemia/reperfusion, 5 min each) significantly reduced the total number of ventricular premature complexes (PVC) and severity of arrhythmias (arrhythmia score; AS) over the whole period of left anterior descending coronary artery occlusion in comparison with non-PC controls (PVC 166±40; AS 1.6±0.2 vs. 550±60 and 3.2±0.2; respectively; P<0.05). In a setting of global ischemia/reperfusion, PC decreased IS (in % of the left ventricle, LV) by 73 %. Pretreatment with wortmannin modified neither arrhythmogenesis nor IS in the non-PC hearts. Bracketing of PC with wortmannin did not abolish antiarrhythmic protection (PVC 92±25; AS 1.7±0.2; P<0.05 vs. non-PC hearts). On the other hand, wortmannin increased IS/LV in the PC hearts to 24±1.2 % as compared with 9 ± 0.6 % in the untreated ones (P<0.05). In conclusion, PI3K/Akt inhibition did not affect reduced arrhythmogenesis during ischemia in the PC hearts indicating that in contrast to its positive role in the irreversible myocardial injury, PI3K/Akt activity is not required for protection induced by PC against ischemic arrhythmias in the rat heart.

### Key words

Myocardial ischemia • Arrhythmias • Preconditioning • PI3-kinase/Akt • Rat heart

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Previous studies demonstrated that phosphatidylinositol 3-kinase (PI3K) and its effector protein kinase B (Akt) is implicated in cell survival (Matsui and Rosenzweig 2005) and that acute PI3K/Akt activation underlies infarct size (IS)-limiting effect of ischemic preconditioning (PC) in the rat heart (Mocanu *et al.* 2002, Hausenloy and Yellon 2006). On the other hand, a selective inhibitor of PI3K/Akt LY294002 that suppressed protection against ischemia/reperfusion injury as documented by attenuation of both anti-infarct effect and improved functional recovery conferred by PC (Mocanu *et al.* 2002, Tong *et al.* 2000) did not abrogate protection against ischemia-induced arrhythmias in these hearts (Ravingerová *et al.* 2007, Matejíková *et al.* 2008a). In all those studies, LY294002 was used in the concentration that blocked PC-induced Akt phosphorylation (Mocanu *et al.* 2002, Tong *et al.* 2000). However, other studies revealed that, independent of PI3K-blocking effect, LY294002 in the same concentration range (5-25 µM) caused inhibition of outward K<sup>+</sup> currents and changes in action potential profile in rat and mouse cardiomyocytes, *via* a direct

**Table 1.** Effect of 15-min perfusion with wortmannin (W) on the preischemic values of heart rate (HR) and occurrence of ventricular tachycardia (VT) during 30-min ischemia in the non-preconditioned (Non-PC) and preconditioned (PC) rat hearts.

Groups	HR (beats/min)	Incidence of VT (%)	Total duration of VT (s)	Number of VT episodes
Non-PC	283 ± 11	100	41.6 ± 8.1	12.1 ± 2.4
Non-PC + W	295 ± 13	88	43.0 ± 6.0	11.2 ± 2.0
PC	286 ± 10	20*	0.7 ± 0.3*	0.2 ± 0.1*
PC + W	280 ± 18	12*	1.2 ± 0.4*	1.1 ± 0.6*

Data are means ± S.E.M. from 10-12 hearts per group. \* P<0.05 vs. non-PC group.

blockade of voltage-dependent K<sup>+</sup> (K<sub>v</sub>) channels (Oudit *et al.* 2004, Sun *et al.* 2004). To discern potential PI3K-independent electrophysiological effects of LY294002 and to verify the differential role of PI3K/Akt in the antiarrhythmic and IS-limiting effects of PC, in the present study we used a structurally and mechanistically distinct (Walker *et al.* 2000) PI3K inhibitor wortmannin.

Male Wistar rats (250-300 g body weight), fed a standard diet and tap water *ad libitum*, were employed. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by US National Institutes of Health (NIH publication No 85-23, revised 1996). After anesthesia (sodium pentobarbitone, 60 mg/kg, *i.p.*), hearts were rapidly excised and perfused at 37 °C in the Langendorff mode at a constant flow of 10 ml/min/g (Asemu *et al.* 2000) or at a constant perfusion pressure of 70 mm Hg for examination of ventricular arrhythmias or myocardial infarction, respectively. The perfusion solution was a modified Krebs-Henseleit buffer gassed with 95 % O<sub>2</sub> and 5 % CO<sub>2</sub> (pH 7.4) containing (in mM): NaCl 118.0; KCl 3.0; MgSO<sub>4</sub> 1.2; NaHCO<sub>3</sub> 25.0; NaH<sub>2</sub>PO<sub>4</sub> 1.18; CaCl<sub>2</sub> 2.5; glucose 5.5 (Matejíková *et al.* 2008b). An epicardial electrogram was registered by means of two electrodes attached to the apex of the heart and the aortic cannula. Left ventricular (LV) pressure was measured by means of a non-elastic water-filled balloon inserted into the LV cavity and connected to a pressure transducer (MLP844, ADInstruments). LV developed pressure, maximal rates of pressure development and fall, heart rate, pressure-rate product and coronary flow were measured during 15-min stabilization and pre-ischemia period for the evaluation of the potential hemodynamic effects of wortmannin (100 nM) applied for 15 min prior to the onset of ischemia. Heart function and arrhythmias were analyzed using PowerLab/8SP Chart 5 software (ADInstruments).

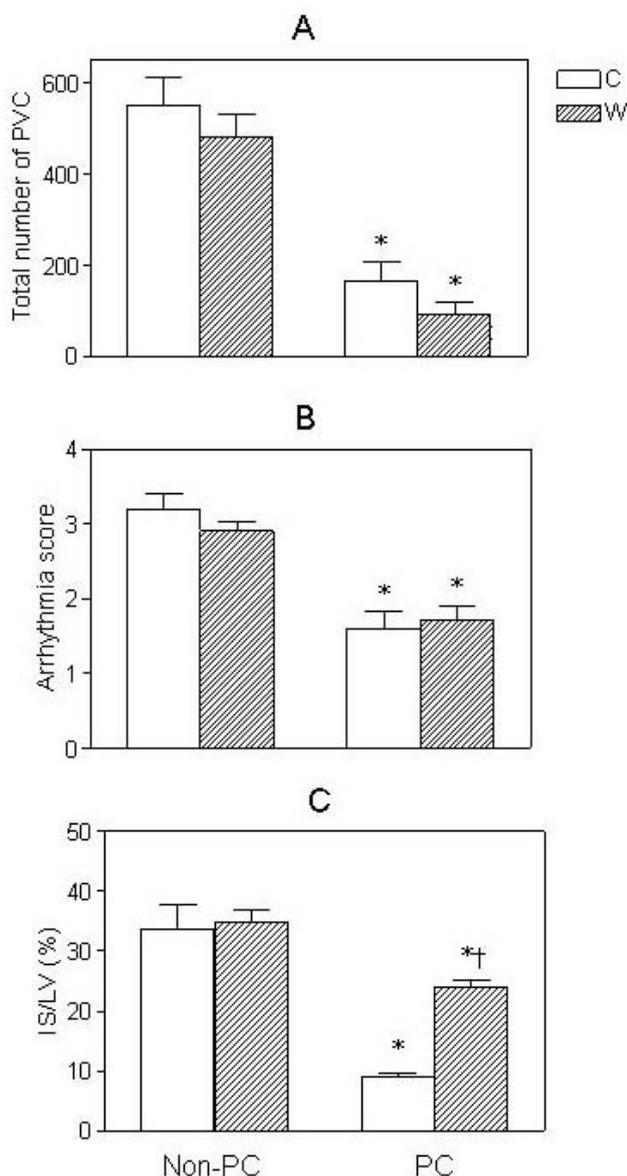
Regional test ischemia was induced by left anterior descending (LAD) coronary artery occlusion for 30 min. In the PC group, the LAD was occluded for 5 min followed by 5-min reperfusion prior to test ischemia. We measured the total number of premature ventricular complexes (PVC) over the whole period of ischemia, the incidence and duration of ventricular tachycardia (VT), and quantified arrhythmia severity using a 5-point scoring system (Curtis 1998).

For the evaluation of IS, in the additional subset of experiments the hearts underwent 30-min global ischemia followed by 2-h reperfusion. In the PC group, test ischemia was preceded by 5-min ischemia followed by 5-min reperfusion. The area of infarct was determined by staining with 2,3,5-triphenyltetrazolium chloride and a computerized planimetric method as described earlier (Ravingerová *et al.* 2007). The IS was expressed as percentage of the LV area.

Wortmannin (Sigma) was dissolved in dimethyl sulfoxide (Lachema, Czech Republic) before being added into the perfusion medium. The final concentration of dimethyl sulfoxide was <0.01 %; control hearts were treated with the same concentration of the solvent.

The data were expressed as means ± S.E.M. One-way ANOVA and subsequent Student-Newman-Keuls test, as well as Mann-Whitney U test and Fisher's exact test were used where appropriate. Differences were considered as significant at P<0.05.

No significant differences in the values of coronary flow and ventricular hemodynamic parameters between PC and control non-PC groups were observed at baseline and after treatment with wortmannin (data not shown). In addition, administration of wortmannin did not cause changes in the heart rate (Table 1) or morphology of epicardial electrogram in any of the groups. A fall in coronary flow by approximately 40 % at the onset of regional ischemia and its increase upon



**Fig. 1.** Effect of wortmannin (W) on ischemia-induced ventricular arrhythmias during 30-min LAD coronary artery occlusion and the size of infarction after 30-min global ischemia and 2-h reperfusion in the non-preconditioned (Non-PC) and preconditioned (PC) rat hearts. **A:** Number of premature ventricular complexes (PVC). **B:** Severity of arrhythmias (arrhythmia score). **C:** Size of myocardial infarction (IS) area expressed in % of the left ventricle (LV) area. Values are means  $\pm$  S.E.M. from 10-12 hearts per group. \*  $P < 0.05$  vs. non-PC group; †  $P < 0.05$  vs. untreated group (C).

reperfusion were similar in all experimental groups and verified the efficacy of coronary artery occlusion.

Myocardial ischemia resulted in a high ectopic activity in the control non-PC group, and VT as the most severe form of arrhythmias occurred in all hearts. In the PC hearts, the total number of PVC was significantly reduced from  $550 \pm 60$  to  $166 \pm 40$  ( $P < 0.05$ ; Fig. 1A). In

addition, the incidence and total duration of VT, as well as the number of its episodes were also markedly decreased (Table 1;  $P < 0.05$  vs. non-PC controls). Accordingly, severity of arrhythmias (arrhythmia score; AS) in these hearts was much lower than in the non-PC group ( $1.6 \pm 0.22$  vs.  $3.2 \pm 0.2$ ;  $P < 0.05$ ; Fig. 1B). Administration of wortmannin did not affect arrhythmogenesis in the non-PC hearts: PVC  $480 \pm 50$ , AS  $2.9 \pm 0.13$ , VT 88 % (Fig. 1A,B; Table 1). Bracketing of PC with wortmannin also failed to reverse its antiarrhythmic effect. Total number of PVC remained significantly lower ( $92 \pm 25$ ;  $P < 0.05$  vs. non-PC group; Fig. 1A). A reduced number of short episodes of VT occurred only in 12 % of wortmannin-treated PC hearts ( $P < 0.05$  vs. non-PC group; Table 1), in which arrhythmia severity was decreased, similar to the untreated PC controls (AS  $1.7 \pm 0.19$ ;  $P > 0.05$  vs. untreated PC group; Fig. 1B).

In the protocol of global ischemia-reperfusion, PC diminished IS from  $33.7 \pm 4.0$  % of LV to  $9.0 \pm 0.6$  % ( $P < 0.05$ ). Administration of wortmannin did not affect IS in the non-PC group (Fig. 1C). In contrast, in the PC hearts, presence of wortmannin abrogated cardioprotective effect and increased IS/LV to  $24.0 \pm 1.2$  % ( $P < 0.05$  as compared with the untreated PC group; Fig. 1C).

It is proposed that acute activation of PI3K/Akt during the preconditioning phase plays an important role in cardioprotection by mediating antiapoptotic effects (Murphy 2004, Uchiyama *et al.* 2004). However, most of the studies are focused on the postischemic phase. In line, our results demonstrate that wortmannin, which in the same protocol of PC blocked an enhanced Akt phosphorylation before the onset of test ischemia (Barančik *et al.* 2008), abolished anti-infarct protection in the PC hearts. On the other hand, while electrophysiological studies established a role of PI3K/Akt in the regulation of membrane ion transport systems that determine membrane repolarization (Steinberg 2001, Gamper *et al.* 2002), the role of this cascade in the antiarrhythmic protection, especially during the ischemic phase of ischemia-reperfusion injury, is less elucidated. PI3K antagonists LY294002 and wortmannin that at low concentrations are considered as selective PI3K inhibitors and valuable tools for the study of cardioprotection (Oudit *et al.* 2004), differ in their structure and mode of action (Walker *et al.* 2000). LY294002 reversibly inhibits PI3K by competing with ATP for the active site of the catalytic subunit, p110 (Davies *et al.* 2000), while wortmannin

irreversibly inactivates PI3K by covalent modification of its catalytic subunit (Yano *et al.* 1993). However, LY294002, but not wortmannin, blocks  $K_v$  currents ( $I_{K,slow}$ ) in mouse ventricular cardiomyocytes (Sun *et al.* 2004) and also directly inhibits  $K_v$  currents ( $K_v2.1$  and  $K_v1.4$ ) in rat pancreatic  $\beta$ -cells and in various cell lines (El-Kholy *et al.* 2003). The channels blocked by LY294002 (including  $K_v1.5$  and  $K_v2.1$ ) are expressed in rodents (Nerbonne 2000) in various cell types. In particular, in rats  $K_v2.1$  is abundant in both pancreatic  $\beta$ -cells (MacDonald and Wheeler 2003) and ventricular cardiomyocytes (Barry *et al.* 1995) that might result in a similar response to pharmacological modulations in these tissues. In mouse and rat ventricular myocytes, LY294002 caused prolongation of action potential duration (Sun *et al.* 2004, Oudit *et al.* 2004) that might be relevant in a setting of acute ischemia and modify the occurrence of reentry arrhythmias. Nevertheless, these PI3K-independent effects do not seem to account for the failure of LY294002 to block antiarrhythmic effects of PC. Wortmannin that does not share  $K^+$  channels blocking activity with LY294002 (El-Kholy *et al.* 2003, Sun *et al.* 2004) and did not affect electrical activity of the heart in the present study, had no influence on reduced arrhythmogenesis as well, although it blocked PC-induced elevation of Akt activation just prior to sustained ischemia (Barančík *et al.* 2008), similar to the effect of LY294002 (Mocanu *et al.* 2002). The latter indicates that PI3K activity is not required for antiarrhythmic effect of PC.

Our results differ from those of Hirata *et al.* (2005) who demonstrated that in the canine myocardium, acutely increased Akt phosphorylation led to suppression

of lethal arrhythmias and limitation of IS, and wortmannin blocked both anti-infarct and antifibrillatory effects. However, that study was focused on the postischemic injury and reperfusion-induced arrhythmias which differ from those induced by ischemia. Thus, besides differences between the species and end-points of protection, the differences between the mechanisms of different types of arrhythmias occurring during ischemia and reperfusion (Curtis and Hearse 1989) should also be taken into consideration when susceptibility to arrhythmias is to be characterized.

In conclusion, bracketing of PC with an irreversible PI3K/Akt inhibitor wortmannin, that abolished protection against lethal myocardial injury, did not reverse protection against ventricular arrhythmias conferred by PC during sustained ischemia, corroborating our previous findings of the LY294002-independent protection against ischemia-induced arrhythmias in the preconditioned rat heart. Thus, taken together our results indicate that enhanced tolerance to ischemic arrhythmias in the PC hearts does not appear to be mediated by PI3K/Akt suggesting that PI3K/Akt activity may play distinct roles in the IS limitation and antiarrhythmic protection.

### Conflict of Interest

There is no conflict of interest.

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