SHORT COMMUNICATION

Role of NO/cGMP signaling pathway in cardiac ischemic tolerance of chronically hypoxic rats

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Key words

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Summary

It has been suggested that increase in acute nitric oxide (NO) or cyclic guanosine monophosphate production may be involved in cardioprotection induced by chronic hypoxia (CH). We studied the effect of NO donor molsidomine and phosphodiesterase type 5 inhibitor sildenafil on myocardial ischemia/reperfusion injury in rats adapted to CH. Male Wistar rats were exposed to continuous hypoxia in a normobaric chamber (10% O_2, 4 weeks). Rats received either saline, molsidomine (10 mg/kg body weight, i.v.) or sildenafil (0.7 mg/kg body weight, i.v.) 30 min before ischemia. Control rats were kept under normoxia and treated in a corresponding manner. Adaptation to CH increased the myocardial ischemic tolerance. Acute treatment with either molsidomine or sildenafil significantly reduced infarct size in normoxic rats and further enhanced cardioprotection induced by CH. However, the cardioprotective effect of CH on ischemia/reperfusion injury was not additive to the cardioprotection provided by the drugs.

Ischemic heart disease belongs to the leading cause of mortality worldwide. Therefore, therapeutic strategies trying to protect the myocardium against ischemia/reperfusion (I/R) injury have been widely studied. Many experimental studies have repeatedly confirmed that heart can be protected from I/R injury by cardioprotective phenomena such as adaptation to chronic hypoxia (CH) or different types of conditioning (Peart and Headrick, 2009). It was demonstrated repeatedly that adaptation to CH can reduce infarct size, number of ischemic arrhythmias and postischemic contractile dysfunction (reviewed by Ošťádal and Kolář, 2007). However, compared to the temporary character of conditioning, these cardioprotective effects may persist for weeks or even months after the cessation of hypoxic exposure (Neckář et al., 2004). Although the beneficial effect of CH on various manifestations of I/R injury has been known for many years, the molecular mechanism remains far from being understood. CH, besides the other changes, increases the production of nitric oxide (NO) in the myocardium (Ding et al., 2005; Fitzpatrick et al., 2005) suggesting that enhanced NO generation plays a role in its protective mechanism. Many beneficial signals of NO are mediated by cyclic guanosine monophosphate (cGMP), produced by soluble guanylate cyclase after NO activation. In the heart, cGMP is degraded by phosphodiesterase type 5 (PDE5; Giordano et al., 2001). Inhibition of PDE5 activity would be then expected to protect the ischemic heart by reducing the breakdown of cGMP in CH hearts.

In the present study, we tested a hypothesis that acute administration of NO donor molsidomine or PDE5 inhibitor sildenafil can further reduce myocardial infarct size in CH rats subjected to I/R injury. Adult male Wistar rats (300-350 g) were exposed to CH (10% O_2) in a normobaric chamber equipped with hypoxic generators for 4 weeks. Control rats were kept for the same period of time at room air. Animals were randomly assigned to six groups; normoxic (n=10) and CH (n=10) rats treated with saline, normoxic (n=11) and CH (n=11) rats treated with molsidomine (Sigma Aldrich, St. Louis, MO, USA; 10 mg/kg body weight, i.v.),

normoxic (n=8) and CH (n=10) rats treated with sildenafil (Viagra, Pfizer, Kent, Great Britain; 0.7 mg/kg body weight, i.v.) 30 min before coronary artery occlusion. Molsidomine and sildenafil doses were selected according to Chander and Chopra (2005) and Koneru et al. (2008), respectively. All experiments were performed in accordance with the Guide for the Care and Use of laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Ethics Committee of the Institute of Physiology, Academy of Sciences of the Czech Republic.

Susceptibility to myocardial injury was evaluated in anesthetized (sodium pentobarbital; 60 mg/kg body weight) open-chest pump-ventilated (68-70 strokes/min, tidal volume of 1.2 ml/100 g body weight) rats. Cannulations of jugular vein (drug administration) and carotid artery (mean arterial pressure recording) were accomplished. Left thoracotomy was performed to expose the heart; myocardial ischemia was induced by occlusion of left anterior descending coronary artery for 20 min, followed by 3-h reperfusion. The area at risk and the infarct area were delineated by staining with potassium permanganate and 2,3,5-triphenyltetrazolium chloride, respectively, and determined by a computerized planimetric method. Detailed description of the experimental procedures is given elsewhere (Neckář et al., 2004). Data are presented as mean values±SEM. GraphPad Prism software was used and statistical evaluations were done using one-way analysis of variance with the Newman-Keuls post test. Values exceeding the 95% probability limits ($P<0.05$) were considered statistically significant.

The values of mean arterial pressure (MAP) in selected time intervals of the experimental protocol are shown in Figure 1. Acute molsidomine administration induced a significant decrease in MAP in both normoxic (from 128.3±4.5 to 56.6±4.6 mmHg; $P<0.05$) and CH (from 147±4.3 to 56.3±3.4 mmHg; $P<0.05$) rats; this effect persisted during ischemia. Acute sildenafil administration did not have lasting effect on MAP in normoxic or CH group.

Figure 2A shows the myocardial area at risk expressed as the percentage of the left ventricular size. No significant differences occurred among the individual experimental groups; this allowed comparing the average values of infarct size. As shown in Figure 2B, adaptation to CH induced a significant decrease in infarct size normalized to the area at risk (40.6±2.4%) as compared to the normoxic controls (56.3±2.8%; $P<0.05$). Acute molsidomine or sildenafil administration markedly reduced myocardial infarct size in normoxic rats to 32.3±6.3% and 33.7±8.4%, respectively ($P<0.05$) and significantly enhanced protective effect of CH (26±2.5% and 24.4±3.5%, respectively; $P<0.05$) in comparison to the saline groups. However, myocardial infarct size in CH rats treated with molsidomine or sildenafil did not differ from treated normoxic animals.

It is well known that adaptation to CH influences myocardial NO production and signaling (Manukhina et al., 2006). As was shown previously, CH increased concentrations of NO markers like cGMP, nitrites and nitrates in cardiovascular system (Manukhina et al., 1999; Shi et al., 2000; Zhong et al., 2002). Indeed, it appears that adaptation to CH increases myocardial expression and activity of NO synthases (NOS) though it remains controversial which isoform is responsible for the increased NO production. Ferreiro et al. (2001) showed higher expression of inducible NOS (iNOS) in right atrium samples of children with hypoxemic congenital heart defects. We and others found higher iNOS expression in left ventricle of adult CH rats (Rouet-Benzineb et al., 1999; Kolář et al., 2003; Grilli et al., 2003). Moreover, Ding et al. (2005) showed that the acute administration of aminoguanidin, the iNOS inhibitor, abolished cardioprotective effect of CH. On the other hand, in immature rabbit hearts CH induced the overexpression of endothelial NOS (eNOS) and the molecules regulating eNOS-dependent NO production such as calveolin 3 and heat shock protein 90 (Baker et al., 1999; Shi et al., 2000 and 2002). Therefore, species (rat vs. rabbit) and

ontogenetic (adult vs. neonatal) differences may account for altered myocardial expression of NOS isoforms in CH hearts.

Baker's group also analyzed ischemic tolerance in CH immature rabbit hearts after acute modulation of NO availability. They found that the acute administration of general NOS inhibitor L-NAME before ischemia completely abolished cardioprotective effect of CH (Baker et al., 1999). Similar findings were also reported for CH immature rat hearts (Ošťádalová et al., 2002). On the other hand, drugs increasing NO/cGMP signaling mimicked cardioprotection in both immature and adult normoxic animals (Nitz and Fiedler, 1987; Baker et al., 1999; Ockaili et al., 2002). These results are in line with the present study demonstrating strong infarct size-limiting effect of molsidomine and sildenafil in adult normoxic rat hearts. Although both molsidomine and sildenafil were able to further reduce infarct size in CH hearts, the combination of both protective measures (CH and molsidomine or sildenafil) did not provide additive protection to that induced by these drugs in normoxic hearts. Therefore, it seems that the activation of protection by drugs increasing NO/cGMP levels was filled to maximal capacity. Similar results were observed when CH was combined with classic ischemic preconditioning (Neckář et al., 2002) suggesting that the cardioprotective interventions (CH and ischemic/pharmacological preconditioning, respectively) share the same signaling pathways (as was reviewed previously by Ošťádal and Kolář, 2007).

In conclusion, the present study showed that acute preischemic treatment with NO donor molsidomine or PDE5 inhibitor sildenafil improved cardiac ischemic tolerance not only in normoxic rats but also in animals adapted to CH. However, cardioprotective effect of CH was not additive to the cardioprotection provided by the drugs. These results suggest the notable role of NO/cGMP signaling pathway in cardioprotection induced by adaptation to CH.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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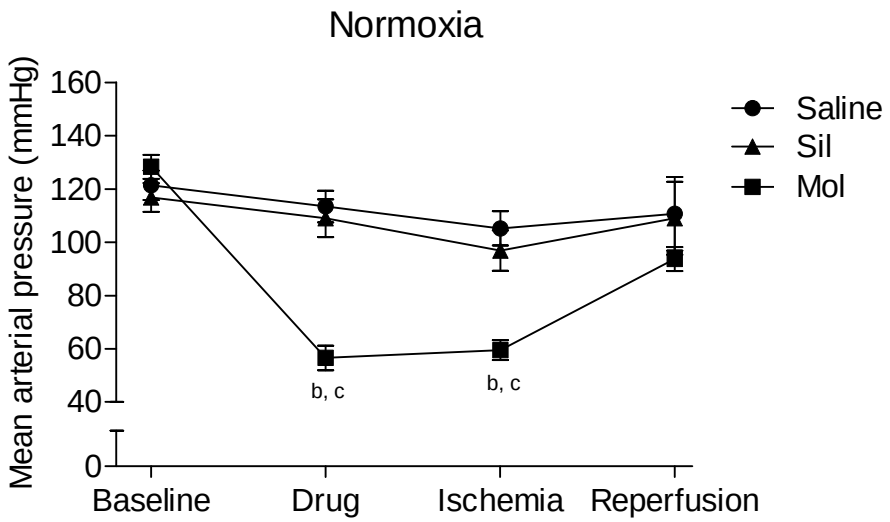
A



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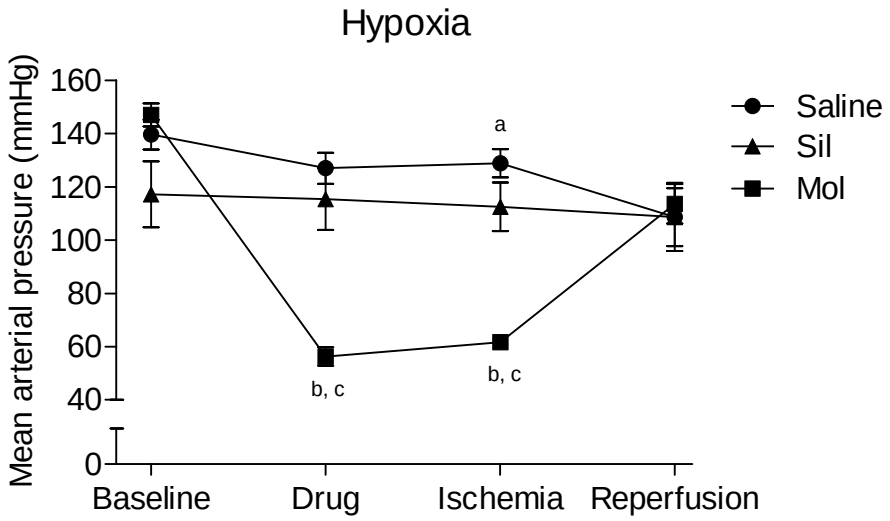
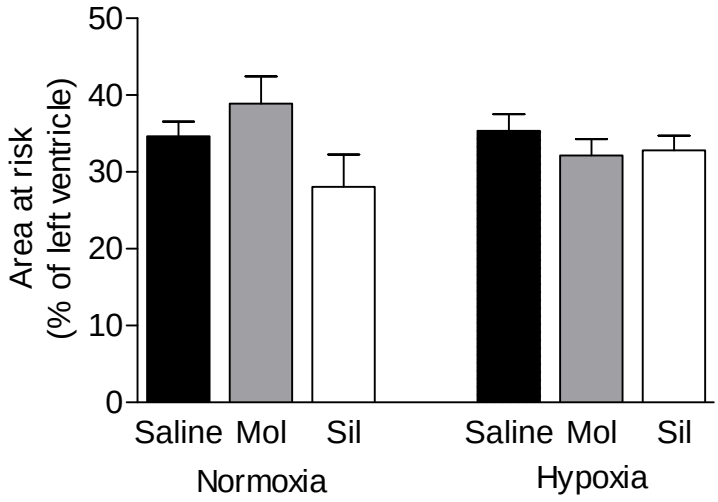


Figure 1 Mean arterial pressure at baseline, after drug administration, at the end of 20-min coronary artery occlusion and at the end of 3-h reperfusion in normoxic (A) and chronically hypoxic (B) rats treated with saline (Saline), molsidomine (Mol) or sildenafil (Sil). Data are expressed as mean±SEM; n=8-11 per group. [a] P<0.05 vs. corresponding normoxic group, [b] P<0.05 vs. corresponding Saline group, [c] P<0.05 vs. Baseline.

A



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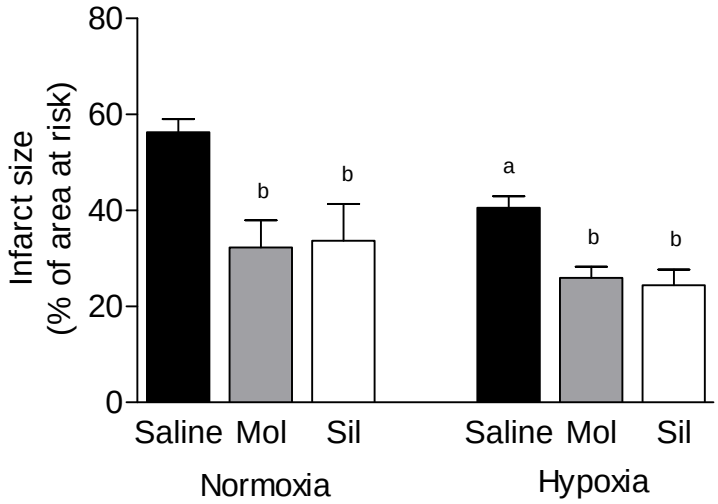

Figure 2 Area at risk expressed as the percentage of left ventricular size (A) and myocardial infarct size expressed as the percentage of area at risk (B) in normoxic and chronically hypoxic rats treated with saline (Saline), molsidomine (Mol) or sildenafil (Sil). Data are expressed as mean±SEM; n=8-11 per group. [a] $P<0.05$ vs. corresponding normoxic group, [b] $P<0.05$ vs. corresponding Saline group.