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RAPID COMMUNICATION

MCC-134, a Blocker of Mitochondrial and Opener of Sarcolemmal ATP-Sensitive K^+ Channels, Abrogates Cardioprotective Effects of Chronic Hypoxia

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Summary

We examined the effect of MCC-134, a novel inhibitor of mitochondrial ATP-sensitive K^+ (mitoK_{ATP}) channels and activator of sarcolemmal ATP-sensitive K^+ (sarcK_{ATP}) channels, on cardioprotection conferred by adaptation to chronic hypoxia. Adult male Wistar rats were exposed to intermittent hypobaric hypoxia (7000 m, 8 h/day, 5-6 weeks) and susceptibility of their hearts to ventricular arrhythmias and myocardial infarction was evaluated in anesthetized open-chest animals subjected to 20-min coronary artery occlusion and 3-h reperfusion on the day after the last hypoxic exposure. MCC-134 was administered intravenously 10 min before ischemia and 5 min before reperfusion in a total dose of 0.3 mg/kg or 3 mg/kg divided into two equal boluses. The infarct size (tetrazolium staining) was reduced from 59.2±4.4 % of the area at risk in normoxic controls to 43.2±3.3 % in the chronically hypoxic group. Chronic hypoxia decreased the reperfusion arrhythmia score from 2.4±0.5 in normoxic animals to 0.7±0.5. Both doses of MCC-134 completely abolished the antiarrhythmic protection (score 2.4±0.7 and 2.5±0.5, respectively) but only the high dose blocked the infarct size-limiting effect of chronic hypoxia (54.2±3.7 %). MCC-134 had no effect in the normoxic group. These results support the view that the opening of mitoK_{ATP} channels but not sarcK_{ATP} channels plays a crucial role in the mechanism by which chronic hypoxia improves cardiac tolerance to ischemia/reperfusion injury.

Key words

Chronic hypoxia • Ischemia/reperfusion • Cardioprotection • MCC-134 • K_{ATP} channels

Chronic hypoxia has been shown to protect the heart against all major manifestations of acute ischemia/reperfusion (I/R) injury by a mechanism involving the activation of ATP-sensitive K^+ (K_{ATP}) channels, in particular those that are localized in the inner mitochondrial membrane (mitoK_{ATP}). This conclusion is based mostly on the results of experiments that examined the effects of specific pharmacological modulators of these channels on cardiac ischemic tolerance in chronically hypoxic rats (for review see Kolář and Ošťádal 2004). In our studies, the mitoK_{ATP} antagonist, 5-hydroxydecanoate (5-HD), completely abolished the infarct size reduction, the improvement of postischemic recovery of contractility, and antiarrhythmic protection in

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chronically hypoxic hearts, but it had no effect in normoxic controls. In addition, mitoK$_{ATP}$ openers, diazoxide or BMS-191095, reduced infarct size, increased the recovery of contractility and decreased the severity of ventricular arrhythmias induced by I/R in normoxic hearts, but no additive protection occurred in the hypoxic group (Asemu *et al.* 1999, Neckář *et al.* 1999, 2002b). MitoK$_{ATP}$ channels also appear to play a role in increased cardiac ischemic tolerance of neonatal rats kept hypoxic for 10 days after birth as the improved postischemic function of their hearts was blocked by 5-HD (Ošťádalová *et al.* 2002). Likewise, 5-HD abrogated the protective effect of chronic hypoxia against cytosolic Ca^{2+} overload induced by acute hypoxia/reoxygenation in rat isolated cardiomyocytes (Zhu *et al.* 2003). Besides long-lasting protection conferred by chronic hypoxia, mechanisms of both early and delayed forms of preconditioning induced by various stimuli also involve mitoK$_{ATP}$ channels as implicated by many reports (for a review see Garlid *et al.* 2003).

Nevertheless, certain controversy still exists concerning the role of K$_{ATP}$ channel subtypes in cardioprotection. The reason is that 5-HD and diazoxide, the common drugs that are generally believed to target only one channel subtype (mitoK$_{ATP}$), might not be sufficiently selective under certain conditions. For example, 5-HD can block and diazoxide can activate also sarcolemmal K$_{ATP}$ (sarcK$_{ATP}$) channels at high ADP level and low pH as well as after metabolic inhibition (Notsu *et al.* 1992, D'hahan *et al.* 1999, Matsuoka *et al.* 2000). In addition, these modulators are apparently not completely specific for K$_{ATP}$ and the involvement of their side effects in cardioprotection cannot be excluded. 5-HD is a derivative of fatty acid decanoate, which can be metabolized by acyl-CoA synthase to 5-HD-CoA and create a rate-limiting bottleneck for β-oxidation of fatty acids, whereas diazoxide is known to inhibit oxidation of succinate and 2-oxyglutarate (Lim *et al.* 2002, Hanley *et al.* 2002, 2005).

Table 1. Heart rate and blood pressure in control and MCC-134-treated rats adapted to IHA hypoxia and in normoxic animals

	Baseline	**Preischemia**	**Ischemia**	**Reperfusion**
Heart rate (beats/min)				
Normoxic	422 ± 8	416 ± 8	420 ± 11	352 ± 13 [b]
Normoxic + MCC 0.3	439 ± 13	445 ± 15 [c]	448 ± 10 [c]	386 ± 12 [b c]
Normoxic + MCC 3	437 ± 10	449 ± 6 [c]	457 ± 5 [c]	392 ± 9 [b c]
Hypoxic	436 ± 7	425 ± 6	428 ± 4	399 ± 7 [a b]
Hypoxic + MCC 0.3	435 ± 4	429 ± 7	427 ± 7	412 ± 11 [b]
Hypoxic + MCC 3	425 ± 9	442 ± 6 [c]	442 ± 8	326 ± 8 [a b c]
Mean arterial pressure (mm Hg)				
Normoxic	115 ± 8	120 ± 6	117 ± 10	80 ± 10 [b]
Normoxic + MCC 0.3	109 ± 7	116 ± 10	125 ± 6	97 ± 9
Normoxic + MCC 3	108 ± 8	116 ± 9	107 ± 5	87 ± 6 [b]
Hypoxic	119 ± 10	121 ± 12	129 ± 7	134 ± 4 [a]
Hypoxic + MCC 0.3	114 ± 10	120 ± 9	130 ± 9	122 ± 9 [a]
Hypoxic + MCC 3	116 ± 8	123 ± 6	120 ± 7	107 ± 5 [a c]

Values are means ± S.E.M. at baseline, after the addition of MCC-134 in a dose of 0.3 mg/kg or 3 mg/kg (preischemia), at the end of 20-min coronary artery occlusion, and at the end of 3-h reperfusion. Each group contained 6-8 rats. [a] $P<0.05$ *vs.* corresponding normoxic group; [b] $P<0.05$ *vs.* baseline; [c] $P<0.05$ *vs.* corresponding untreated group.

The recently developed vasorelaxing agent, MCC-134 (1-[4-(1*H*-imidazol-1-yl)benzoyl]-*N*-methyl-cyclobutanecarbothioamide), could be a useful pharmacological tool in solving this controversy as it has unique properties. It acts as a blocker of mitoK$_{ATP}$ channels and opener of sarcK$_{ATP}$ channels (Sasaki *et al.* 2003). We, therefore, examined the effects of this agent on cardiac ischemic tolerance in chronically hypoxic and control normoxic rats.

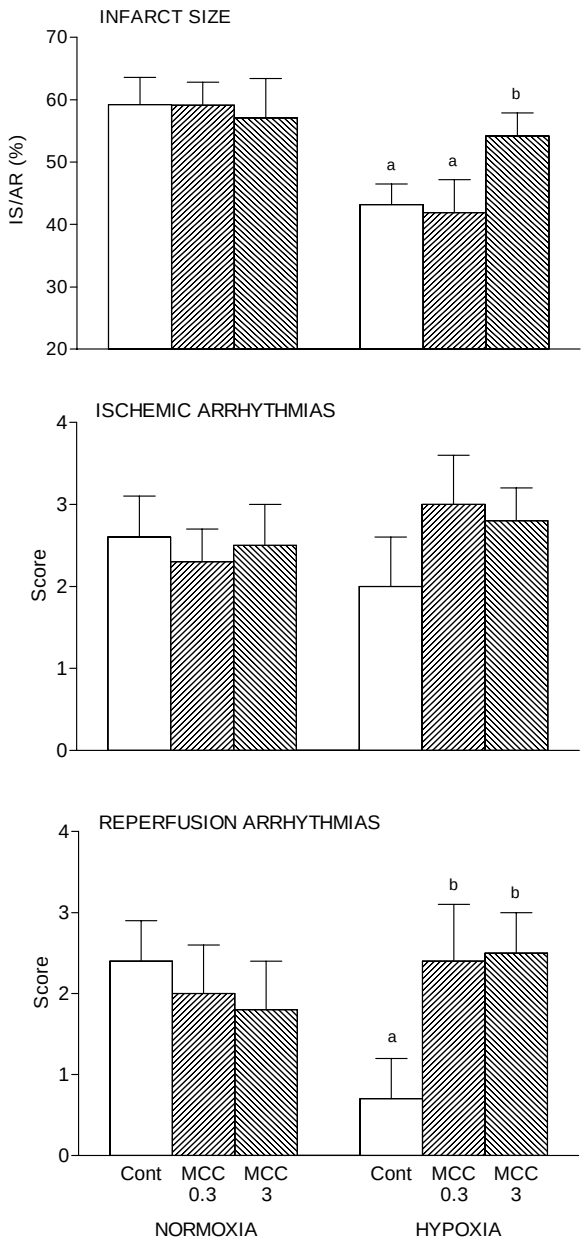

Fig. 1. Myocardial infarct size expressed as a percentage of the area at risk (IS/AR, upper panel) and ventricular arrhythmia score over 20-min ischemia (middle panel) and early reperfusion (lower panel) in control (Cont) and MCC-134-treated rats adapted to IHA hypoxia and in normoxic animals. MCC-134 was administered in a dose of 0.3 mg/kg (MCC 0.3) or 3 mg/kg (MCC 3). Values are means ± SEM; [a] $P<0.05$ *vs.* corresponding normoxic group; [b] $P<0.05$ *vs.* corresponding untreated control group.

Adult male Wistar rats were adapted for 5-6 weeks to intermittent high altitude (IHA) hypoxia in a hypobaric chamber (7000 m, 8 hours/day). Susceptibility of their hearts to ventricular arrhythmias and myocardial infarction was evaluated in anesthetized (sodium pentobarbitone; 60 mg/kg body weight) open-chest pump-ventilated (68 strokes/min, tidal volume of 12 ml/kg body weight) animals subjected to I/R insult on the day after the last hypoxic exposure. MCC-134 dissolved in DMSO was administered intravenously 10 min before ischemia and 5 min before reperfusion in a total dose of 0.3 mg/kg or 3 mg/kg divided into two equal boluses; control animals received the same volume of the solvent. A single-lead electrocardiogram and blood pressure in the carotid artery were continually recorded. Regional myocardial ischemia was induced by occlusion of the left anterior descending coronary artery for 20 min, followed by 3-h reperfusion. The incidence and severity of ischemic and reperfusion ventricular arrhythmias was evaluated using a 5-point arrhythmia score. 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 model, methods and statistical analysis is given elsewhere (Neckář *et al.* 2002a, 2004).

Baseline values of heart rate and mean arterial pressure did not appreciably differ between the groups. Both variables were better preserved at the end of reperfusion in chronically hypoxic rats than in normoxic ones and the high dose of MCC-134 tended to reduce these differences (Table 1). IHA hypoxia significantly decreased infarct size as compared with the normoxic group and this protective effect was completely abolished by the high dose of MCC-134 (Fig. 1); the area at risk normalized to the size of the left ventricle did not significantly differ between the groups. Moreover, IHA hypoxia significantly decreased the arrhythmia severity, expressed as arrhythmia score, during early reperfusion and MCC-134 abrogated this antiarrhythmic protection (Fig. 1); effects on ischemic arrhythmia score were not significant. MCC-134 did not influence cardiac ischemic tolerance in normoxic animals.

These results are in line with our previous observations concerning the inhibitory effects of conventional mitoK_{ATP} blocker 5-HD on the increased tolerance of chronically hypoxic rat hearts to I/R injury (Neckář *et al.* 2002b). Both agents, MCC-134 and 5-HD, completely abolished cardioprotection induced by IHA hypoxia despite their distinct chemical structures. MCC-134 is a derivative of aprikalim (Morita *et al.* 1999) with opposite actions on mitoK_{ATP} and sarcK_{ATP} channel subtypes. Similarly as 5-HD, it blocks mitoK_{ATP} channels but, in addition, it opens sarcK_{ATP} channels. As recently demonstrated, this agent abolished the protective effect of

diazoxide in rabbit and mouse ventricular myocytes in a cell-pelleting model of ischemia and attenuated the effect of ischemic preconditioning against myocardial infarction in mice *in vivo*; these observations argue for the primacy of mitoK_{ATP} channels in the mechanism of preconditioning (Sasaki *et al.* 2003). Complete abrogation of infarct size-limiting and antiarrhythmic protective effects of IHA hypoxia by MCC-134 in the present study further strengthens our previous conclusion (Asemu *et al.* 1999, Neckář *et al.* 2002b) that mitoK_{ATP} channels but not sarcK_{ATP} channels play a crucial role also in the mechanism by which chronic hypoxia in rats improves cardiac tolerance to I/R injury. It appears unlikely that the proarrhythmic action of MCC-134 in chronically hypoxic animals resulted from sarcK_{ATP} opening because a non-selective K_{ATP} blocker, glibenclamide, abolished the antiarrhythmic protection as well (Asemu *et al.* 1999).

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