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Original Article

Milrinone-Induced Postconditioning Requires Activation of Mitochondrial Ca^{2+}-sensitive Potassium (mBK_{Ca}) Channels

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Objectives: Cardioprotection by postconditioning requires activation of mitochondrial large-conductance Ca^{2+}-sensitive potassium (mBK_{Ca}) channels. The involvement of these channels in milrinone-induced postconditioning is unknown. The authors determined whether cardioprotection by milrinone-induced postconditioning involves activation of mBK_{Ca} channels in the rat heart in vitro.
Design: Randomized, prospective, blinded laboratory investigation.
Setting: Experimental laboratory.
Participants: Male Wistar rats.
Interventions: Hearts of male Wistar rats were randomized, placed on a Langendorff system, and perfused with Krebs-Henseleit buffer at a constant pressure of 80 mmHg. All hearts were subjected to 33 minutes of global ischemia and 60 minutes of reperfusion. At the onset of reperfusion, hearts were perfused with different concentrations of milrinone (0.3-100 μM) for determination of a dose-effect curve. In a second set of experiments, 3 μM milrinone was administered in combination with the mBK_{Ca} channel inhibitor paxilline (1 μM). Infarct size was determined by triphenyltetrazoliumchloride staining.
Measurements and Main Results: In control animals, infarct size was 37 ± 7%. Milrinone at a concentration of 3 μM reduced infarct size to 22 ± 7% ($p < 0.05$ *v* control). Higher milrinone concentrations did not confer stronger protection. Paxilline completely blocked milrinone-induced cardioprotection whereas paxilline alone had no effect on infarct size.
Conclusions: This study shows that activation of mBK_{Ca} channels plays a pivotal role in milrinone-induced postconditioning.

Key Words: milrinone; postconditioning; reperfusion injury; myocardial infarction

AFTER A MYOCARDIAL INFARCTION has occurred, restoration of perfusion is crucial for survivability. However, 50% of the final infarct size is caused by reperfusion itself;

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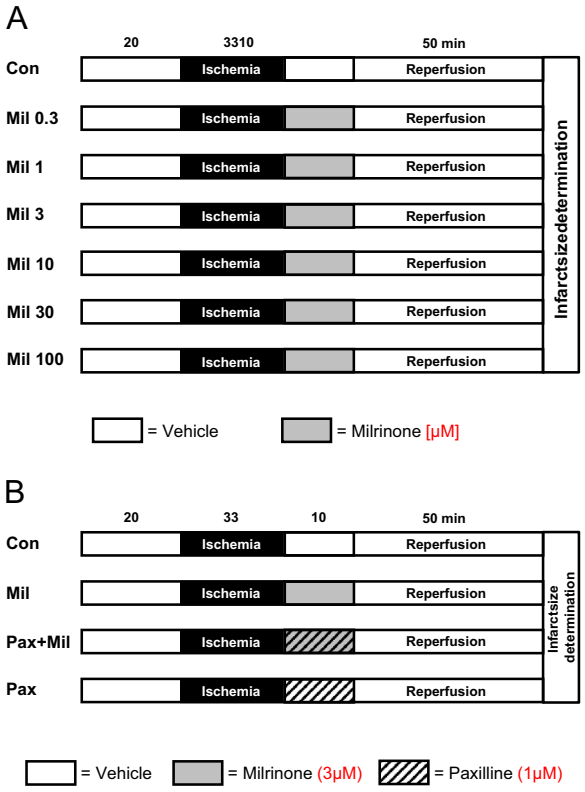

Fig 1. Experimental protocol. (A) Part 1 of the study. (B) Part 2 of the study. Con, control; Mil, milrinone; Pax, paxilline.

thus, reperfusion paradoxically reduces the beneficial effects of restored coronary blood flow.[1] Preconditioning and postconditioning are strategies that have been applied successfully to protect the heart against the consequences of myocardial ischemia and reperfusion injury.[2]

The phosphodiesterase-3-inhibitor milrinone (Mil) is indicated for the treatment of acute heart failure and cardiogenic shock.[3] By inhibiting phosphodiesterase-3, Mil leads to an increase of the second messenger cyclic adenosine monophosphate (cAMP), resulting in phosphorylation of protein kinases and activation of calcium channels.[4] Mil has been shown to exert cardioprotective properties and to protect against myocardial ischemia-reperfusion injury.[4] This effect of Mil is thought to be mediated by inhibiting cAMP, leading to phosphorylation of protein kinase A (PKA) and mitogen-activated protein kinase (MAPK).[4] However, the exact underlying molecular mechanism of Mil-induced cardioprotection is unknown and remains to be determined.

Activation of large-conductance Ca^{2+}-sensitive potassium (BK_{Ca}) channels localized on the inner mitochondrial membrane of cardiomyocytes[5] previously has been described as essential in the signaling pathway of several cardioprotective interventions.[6–9] The authors hypothesized that Mil-induced postconditioning involves activation BK_{Ca} channels.

Methods

After approval from the Animal Ethics Committee of the University of Düsseldorf, Germany, the study was performed in agreement with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication number 85-23, revised 1996).

Surgical Preparation

The authors performed surgical preparation as specified previously.[9] In brief, the authors injected the rats with pentobarbital (90 mg/kg) intraperitoneally for anesthesia. Subsequently, rats were thoracotomized for excision of the hearts. The hearts were mounted on a Langendorff system and were perfused at constant pressure (80 mmHg) with a Krebs-Henseleit solution at 37°C.[6]

The authors inserted a fluid filled balloon into the left ventricle and set the end-diastolic pressure at 1 to 4 mmHg. For 20 minutes, the hearts were subjected to an equilibration period. The authors detected a heart rate, left ventricular end-systolic pressure (LVESP), rate pressure product (RPP), left ventricular end-diastolic pressure (LVEDP), and coronary flow continuously. They measured the maximal contracture and the time point of maximal contracture in each experiment during ischemia, which is defined as the point of maximal pressure during ischemia and indicates the degree of myocardial injury.[6]

Table 1
Weights and Ischemic Contracture

	n	Body Weight (g)	Heart Weight Dry (g)	Heart Weight Wet (g)	Time of Max. Ischemic Contracture (min)	Level of Max. Ischemic Contracture (mmHg)
Con	10	287 ± 29	0.16 ± 0.02	1.60 ± 0.23	13 ± 2	65 ± 5
Mil 0.3	10	285 ± 24	0.16 ± 0.02	1.46 ± 0.14	14 ± 1	62 ± 6
Mil 1	10	286 ± 24	0.17 ± 0.02	1.56 ± 0.08	13 ± 2	63 ± 6
Mil 3	10	278 ± 25	0.17 ± 0.03	1.48 ± 0.12	13 ± 2	66 ± 8
Mil 10	10	296 ± 32	0.16 ± 0.01	1.62 ± 0.11	14 ± 2	62 ± 9
Mil 30	10	282 ± 23	0.16 ± 0.02	1.55 ± 0.12	13 ± 1	67 ± 10
Mil 100	10	281 ± 21	0.15 ± 0.01	1.49 ± 0.10	13 ± 1	66 ± 7

NOTE. Data are mean ± standard deviation.
Abbreviations: Con, control; Mil, milrinone.

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