Ketamine Modulates Calcium Homeostasis in Hypoxia-Reoxygenated Cardiomyocytes

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Abstract: Ketamine is clinically used as an inducer of anesthesia in critically ill patients because it has more stable hemodynamics than barbiturates or inhaled anesthetic agent. It has been known the effect of anesthetic related with calcium homeostasis in myocardium but there are few studies for myocardial protection of ketamine from ischemia-reperfusion injury. We therefore observed protective effects of ketamine on survival of ischemia-reoxygenated cardiomyocytes in phosphorylation levels of Erk and Akt as well as suppression of pro-apoptotic proteins, Bax and cytochrome C, and induction of anti-apoptotic protein, Bcl-2. Ketamine also overcame intracellular Ca2+ overload. We observed significant induction in transcript level of calreticulin, PMCA1, ion channels(L-type Ca2+-channel, Kir3.4, Kir6.1) and suppression in transcript level of calmodulin, and SERCA 2a in ketamine-treated cardiomyocytes. In conclusion, ketamine was protective of cardiomyocytes under hypoxia-reperfusion condition. Therefore, we have provided new insight into myocardial protection of anesthetic agents so a better understanding of the role of anesthetics in the prevention of myocardial injury may provide strategies to improve outcome.

Key words: calcium homeostasis, cardiomyocytes, hypoxia-reoxygenation, ketamine

1. Introduction

Ketamine is clinically used as an inducer of anesthesia in critically ill patients because it has more stable hemodynamics than barbiturates or inhaled anesthetic agent. Especially, ketamine is frequently used in infant and toddlers for elective surgeries due to its short acting and rapid dissociative anesthesia followed by rapid recovery.1 Cytochrome P450(CYP3A4, CYP2B6, and CYP2CP) is involved in metabolism of ketamine and N-desmethylketamine, the main metabolite of ketamine, may contribute to the analgesic effects following ketamine administration.2 Among perioperative injuries, myocardial ischemia frequently occurs. Because myocardial ischemia-reperfusion injury can lead to severe complications, measures to minimize myocardial damage have been an important target of research.

Previous studies have shown that intravenous anesthesia makes an effect on cardiac parameters such as heart rate and cardiac output of patients.3 The protection by volatile anesthetics is relatively well investigated but there are still some arguments for the cardioprotective effect of ketamine. It is generally agreed that these agents reduce the myocardial damage caused by ischemia and reperfusion.4-6 There are some proposed mechanisms for myocardial protection by anesthetic agents: ischemic preconditioning-like effect, interference in the neutrophil/platelet-endothelium interaction, blockade of Ca2+ overload to the cytosolic space and antioxidant-like effect.7 It was known that some anesthetic could change the expression of proteins related to regulation of cardiac calcium homeostasis in these protective mechanisms.8-10

The pivotal role of calcium cycling and homeostasis has long been recognized in contractile, metabolic, electrical and ionic alterations associated with myocardial ischemia and anoxia, as well as in hibernation, stunning and ischemia and mitochondrial dysfunction associated with reperfusion.11,12 Ischemia and
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Simulated ischemic conditions cause Ca²⁺ overload in the myocardium and the increase in intracellular calcium activates a number of cytosolic proteins, including phospholipases, protein kinases, proteases and endonucleases. Activation of these proteases could lead to proteolysis of proteins involved in the regulation of intracellular calcium levels and, thus, to acceleration of heart damage.

But there are rarely few studies for myocardial protection of ketamine from ischemia-reperfusion injury. The goal of the current study was to determine whether ketamine protects cardiomyocyte from ischemia-reperfusion injury. Ketamine-induced myocardial protection in neonatal rat ventricular cardiomyocyte was characterized further by examining the change of protein related to calcium homeostasis and survival signals.

2. Materials and Methods

2.1 Isolation of Neonatal Rat Cardiomyocytes

Neonatal rat cardiomyocytes were isolated and purified by previously described methods. Briefly, hearts of 1-2 day-old Sprague Dawley rat pups was dissected, and the ventricles were washed with Dulbecco's phosphate-buffered saline solution (pH 7.4, Gibco BRL) lacking Ca²⁺ and Mg²⁺. Using micro-dissecting scissors, hearts was minced until the pieces are approximately 1mm³ and treated with 10 mL of collagenase I (0.8 mg/mL, 262 units/mg, Gibco BRL) for 15 minutes at 37°C. The supernatant was then removed and the tissue was treated with fresh collagenase I solution for an additional 15 minutes. The cells in the supernatant were transferred to a tube containing cell culture medium (α-MEM containing 10% fetal bovine serum, Gibco BRL). The tubes were centrifuged at 1200 rpm for 4 minutes at room temperature, and the cell pellet was resuspended in 5 mL of cell culture medium. The above procedures were repeated 7-9 times until little tissue was left. Cell suspensions were collected and incubated in 100 mm tissue culture dishes for 1-3 hours to reduce fibroblast contamination. The non-adherent cells were collected and incubated with 0.1 µM BrdU added. Cells were then cultured with 10% (v/v) FBS in a CO₂ incubator at 37°C.

2.2 Cell Viability Assay

Cardiomyocytes were plated in triplicate wells of 96 well plates at a density of 1×10⁴ per well, and put in a hypoxic chamber for 1 hour prior to exposure to reperfusion with 0.01-100 µM ketamine for 5 hours. Culture plates containing cardiomyocytes in α-MEM were subjected to hypoxic stress in an anaerobic chamber (ThermoForma) maintained at 37°C in which ambient oxygen was replaced with a mixture of 5% CO₂, 5% H₂ and 90% N₂. Cell viability was determined by the MTT assay. After the incubation period, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) was added to each well to a final concentration of 0.5 mg/mL, and the cells were incubated at 37°C for 3 hours to allow MTT reduction. Formazan crystals were dissolved by adding dimethylsulfoxide (DMSO) and absorbance was measured at the 570 nm with a spectrophotometer.

2.3 Immunoblot Analysis

Cardiomyocytes were put in a hypoxic chamber for 1 hour prior to exposure to reperfusion with 10 µM ketamine for 5 hours. Cells were washed once in PBS and lysed in a lysis buffer (Cell signaling) containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycero phosphate, 1 mM Na₃VO₄, 1 mg/mL leupeptin and 1 mM PMSF. Protein concentrations were determined using the Bradford protein assay kit (BioRad). Proteins were separated in a 12% SDS-polyacrylamide gel and transferred to PVDF membrane (Millipore). After blocking the membrane with Tris-buffered saline-Tween 20 (TBS-T, 0.1% Tween 20) containing 5% non-fat dried milk for 1 hour at room temperature, membranes were washed twice with TBS-t and incubated with primary antibodies for 1 hour at room temperature or for overnight at 4°C. The following primary antibodies were used: rabbit anti-ERK, mouse anti-phospho ERK, mouse anti-Bcl-2, mouse anti-Cytochrome C (Santa Cruz Biotechnology), rabbit anti-Akt, mouse anti-phospho Akt (Cell signaling), rabbit anti-Bax (Assay Designs) and mouse anti-β-actin antibodies (Sigma). The membrane was washed three times with TBS-t for 10 minutes, and then incubated for 1 hour at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies. After extensive washing, the bands were detected by enhanced chemiluminescence (ECL) reagent (Santa Cruz Biotechnology). The band intensities were quantified using a Photo-Image System (Molecular Dynamics).

2.4 Confocal Microscopy and Fluorescence Measurements

The measurement of cytosolic free Ca²⁺ was performed by confocal microscopy analysis. Neonatal rat cardiomyocytes