Expression of calcium homeostasis proteins and protective effects in ketamine-treated cardiomyocytes

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Expression of calcium homeostasis proteins and protective effects in ketamine-treated cardiomyocytes

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The graduate course that I started as I was realizing willingness itself doesn’t make a good doctor for the patients has come to an end to get results now. With the help of several teachers around me, I have completed a course after going through a process of trial and error. And yet I am firmly determined on becoming a doctor who keeps researching even further.

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I am dedicating this thesis with love to my wife, Jee In, who has always

supported me with her unfailing faith and two sons, Seung Won and Hyung Won.

Jang Ho Roh

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ABSTRACT

Expression of calcium homeostasis proteins and protective effects in ketamine-treated cardiomyocytes

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Ketamine is clinically used as an inducer of anesthesia in critically ill patients because it has more stable hemodynamics than barbiturates or inhaled anesthetic agents. There are rarely few studies for the effects of anesthetics related with calcium homeostasis in myocardium and myocardial protection of ketamine from hypoxia-reoxygenation injury.

The purpose of the present study was to investigate the changes in survival proteins, Erk and Akt, apoptosis-related proteins, Bcl-2, Bax, and cytochrome C, and calcium ion (Ca^{2+}) overload in hypoxia-reoxygenated cardiomyocytes treated with ketamine. It was also investigated to compare experimental findings with the results from the gene expression of Ca^{2+}-handling proteins and various ion channels as well as ion

exchangers.

It was found that ketamine had a protective effect on the survival of hypoxia-reoxygenated cardiomyocytes in phosphorylation levels of Erk and Akt as well as on the suppression of pro-apoptotic proteins, Bax and cytochrome C, and induction of anti-apoptotic protein, Bcl-2. Ketamine also overcame the intracellular Ca^{2+} overload. Ketamine induced a significant increase in the transcript level of Ca^{2+}-handling proteins (calsequestrin, calreticulin), ion channels (L-type voltage dependent Ca^{2+}-channel, inward rectifying K^+ channel (K_{ir}) 3.4, K_{ir} 6.1) and suppressed the transcript level of ion exchangers (sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase 2a) in hypoxia-reoxygenated cardiomyocytes.

In conclusion, ketamine increases the survival of cardiomyocyte in hypoxia-reoxygenation, and this protective effect seems to be related to the change in the expression of the proteins regulating the intracellular calcium level. Therefore ketamine may be beneficial to myocardial protection from hypoxic injury and clinical research into ketamine and its protective effect should be performed for practical application.

Key words : cardiomyocyte, calcium homeostasis, ketamine, myocardial protection

Expression of calcium homeostasis proteins and protective effects in ketamine-treated cardiomyocytes

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I. 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 agents.[1] Especially, ketamine is frequently used in infant and toddlers for elective surgeries due to its short acting and rapid dissociative anesthesia followed by a rapid recovery.[2] Cytochrome P450 (CYP3A4, CYP2B6, and CYP2C9) is involved in metabolism of ketamine and N-desmethylketamine (norketamine), the main metabolite of ketamine, may contribute to the analgesic effects following ketamine administration.[3-5]

Myocardial ischemia is one of the most serious complications in perioperative period and needs proper treatment to prevent cardiovascular crisis. In many studies,

strategies 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.[6] Myocardial protection by volatile anesthetics, morphine and propofol is relatively well investigated. It is generally agreed that these agents reduce the myocardial damage caused by hypoxia and reoxygenation.[7] There are some proposed mechanisms for myocardial protection by anesthetic agents: ischemic preconditioning-like effect, interference in the neutrophil/platelet-endothelium interaction, blockade of calcium ion (Ca^{2+}) overload to the cytosolic space and antioxidant-like effect.[8-11]

The pivotal role of calcium cycling and homeostasis has long been recognized in contractile, metabolic, electrical and ionic alterations associated with myocardial ischemia, as well as in hibernation, stunning, and mitochondrial dysfunction associated with reperfusion.[12,13] Ischemia and simulated ischemic conditions cause Ca^{2+} 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 can lead to proteolysis of proteins involved in the regulation of intracellular calcium levels and, thus, to acceleration of heart damage.

There are rarely few studies for the effects of anesthetics related with calcium homeostasis in myocardium and myocardial protection of ketamine from hypoxia-reoxygenation injury. The goal of the current study was to determine whether

ketamine protected cardiomyocyte from hypoxia-reoxygenation injury. Ketamine-induced myocardial protection in neonatal rat ventricular cardiomyocyte was characterized further by examining the change of proteins related to calcium homeostasis and survival signals.

II. MATERIALS AND METHODS

1. Isolation of neonatal rat cardiomyocytes

Neonatal rat cardiomyocytes were isolated and purified by the means described below. Briefly, hearts of 1 or 2 day-old Sprague Dawley rat pups were dissected, and the ventricles washed with Dulbecco's phosphate-buffered saline solution (PBS, pH 7.4, Gibco BRL, Gaithersburg, MD, USA) lacking Ca^{2+} and Mg^{2+}. Using micro-dissecting scissors, hearts were minced until the pieces were approximately 1 mm^3 and treated with 10 ml of collagenase I (0.8 mg/ml, 262 units/mg, Gibco BRL, Gaithersburg, MD, USA) for 15 min at 37°C. The supernatant was then removed and the tissue was treated with fresh collagenase I solution for an additional 15 min. The cells in the supernatant were transferred to a tube containing cell culture medium (α-MEM containing 10% fetal bovine serum, Gibco BRL, Gaithersburg, MD, USA). The tubes were centrifuged at 1200 rpm for 4 min 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 there little tissue was left. Cell suspensions were collected and incubated in 100 mm tissue culture dishes for 1-3 hr to reduce fibroblast contamination. The non-adherent cells were collected and seeded to achieve a final concentration of 5 x 10^5 cells/ml. After incubation for 4-6 hr, the cells were rinsed twice with cell culture medium and 0.1 mM BrdU added. Cells were then cultured in a CO_2 incubator at 37°C.

2. Proliferation assay

Cardiomyocytes were plated in triplicate wells of 96 well plates at a density of 1 x 10^4 per well, and put in hypoxic chamber for 1 hr prior to exposure to repefusion with 0, 0.01, 0.1, 1.0, 10, 100 μM ketamine for 5 hr. Culture plates containing cardiomyocyte in α-MEM were subjected to hypoxic stress in an anaerobic chamber (ThermoForma, Marietta, OH, USA) maintained at 37℃ in which ambient oxygen was replaced by a mixture of 5% CO_2, 5% H_2 and 90% N_2.

The cell viability was determined by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. After the incubation period, MTT solution (Sigma, St. Louis, MO, USA) was added to each well to a final concentration of 0.5 mg/ml and was incubated at 37℃ for 3 hr to allow MTT reduction. The formazan crystals were dissolved by adding dimethylsulfoxide (DMSO) and absorbance was measured at the 570 nm with a spectrophotometer.

3. Immunoblot analysis

Protein-treated cells were washed once in PBS and lysed in a lysis buffer (Cell signaling, Beverly, MA, USA) containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 mg/ml leupeptin, and 1 mM PMSF.

Protein concentrations were determined using the Bradford protein assay kit (BioRad, Hercules, CA, USA). Proteins were separated in a 12% SDS-

polyacrylamide gel and transferred to PVDF membrane (Millipore Co, Bedford, MA, USA). After blocking the membrane with Tris-buffered saline-tween 20 (TBS-T, 0.1% tween 20) containing 5% non-fat dried milk for 1 hr at room temperature, membrane was washed twice with TBS-T and incubated with primary antibodies for 1 hr at room temperature or for overnight at 4°C. The membrane was washed three times with TBS-T for 10 min, and then incubated for 1 hr 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, Santa Cruz, CA, USA). The band intensities were quantified using Photo-Image System (Molecular Dynamics, Uppsala, Sweden).

4. Confocal microscopy and fluorescence measurements

The measurement of cytosolic free Ca^{2+} concentration was performed by the confocal microscopy analysis. Neonatal rat cardiomyocytes were plated on glass coverslips coated with laminin (5 mg/cm^2) for 1 day in cell culture medium (α-MEM containing 10% fetal bovine serum, Gibco BRL, Gaithersburg, MD, USA) and 0.1 μM BrdU. After incubation, the cells were washed with modified Tyrode's solution containing: 0.265 g/l $CaCl_2$, 0.214 g/l $MgCl_2$, 0.2 g/l KCl, 8.0 g/l NaCl, 1 g/l glucose, 0.05 g/l NaH_2PO_4, and 1.0 g/l $NaHCO_3$.

Cells were then loaded with 5 mM of the acetoxymethyl ester of Fura-2 (Fura-2

AM, Molecular Probes, Eugene, OR, USA) for 20 min, in the dark and at room temperature, by incubation in modified Tyrode's solution. Fluorescence images were obtained using an argon laser confocal microscope (Leica, Solms, Germany). Fluorochrome was excited by 488 nm line of argon laser and emitted light was collected through a 510-560 nm bandpass filter. Relative changes of free intracellular Ca^{2+} were determined by measuring fluorescent intensity.

5. Caspase3 assay

The relative caspase3 activity was determined using ApopTarget™ Capase3 Colorimetric Protease Assay, according to manufacturer's instructions (Biosource, Camarillo, CA, USA). This assay is based on the generation of free DEVD-*p*NA chromophore when the provided substrate is cleaved by caspase3. Upon cleavage of the substrate by caspase3, free *p*NA light absorbance can be quantified using a microplate reader at 405 nm.

Briefly, the cultured neonatal cardiomyocytes (3×10^6) after different treatments were harvested in lysis buffer (1M DTT), and cell extracts were centrifuged to eliminate cellular debris. Protein concentration was determined by the Bradford assay (Bio-Rad, Hercules, CA, USA). Aliquots (50 µl) of the cell extracts were incubated at 37°C for 2 hr in the presence of the chromophore substrate. Free DEVD- *p*NA is determined colorimetrically. The comparison of absorbance of *p*NA from apoptotic sample with uninduced control allow determination of the fold increase in Capase3 activity.

6. RT-PCR analysis

The expression levels of various proteins were analyzed by the reverse transcription polymerase chain reaction (RT-PCR) technique. For the RNA preparation, confluent rat neonatal cardiomyocytes were cultured for 48 hr in serum-free α-MEM. Total RNA was prepared by UltraspectTM-II RNA system (Biotecx Laboratories, Houston, TX, USA) and single-stranded cDNA was then synthesized from isolated total RNA by Avian Myeloblastosis virus (AMV) reverse transcriptase. A 20 μl reverse transcription reaction mixture containing 1 μg of total RNA, 1X reverse transcription buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1 mM deoxynucleoside triphosphates (dNTPs) 0.5 unit of RNase inhibitor, 0.5 μg of oligo(dT)15, and 15 units of AMV reverse transcriptase was incubated at 42℃ for 15 min, heated to 99℃ for 5 min, and then incubated at 0-5℃ for 5 min.

PCRs were performed for 35 cycles with 3' and 5' primers based on the sequences of various genes. The actin primers (5'-catcacatactcacaacgctcaac-3' and 5'-catagcacgatggtcgattgtcgt-3') were used as the internal standard. The signal intensity of the amplification product was normalized to its respective actin signal intensity.

7. Statistical analysis

Data are presented as mean ± S.E.M. of more than three separate experiments performed in triplicates. Where the results of blots are shown, a representative experiment is depicted. Comparisons between the multiple groups were performed

with one-way ANOVA (Analysis of Variance) with Bonferroni's test. The statistical significance was defined as $p < 0.05$ and $p < 0.01$.

III. RESULTS

1. The effect of ketamine on survival of hypoxia-reoxygenated cardiomyocytes

Following 5 hr of reoxygenation, the viability of hypoxia-reoxygenated cardiomyocytes was about 50% of the control level. But the survival rate of hypoxia-reoxygenated cardiomyocyte treated with ketamine was more increased than that of the cells without ketamine (Figure 1). The concentration of ketamine largely influenced the survival of hypoxia-reoxygenated cardiomyocyte. The result showed that ketamine had a protective effect on hypoxia-reoxygenated cardiomyocte over 10 μM. Although ketamine improved the viability of hypoxia-reoxygenated cardiomyocyte, the ability to protect cell from hypoxia-reoxygenation might be incomplete because the increasing survival rate was not high.

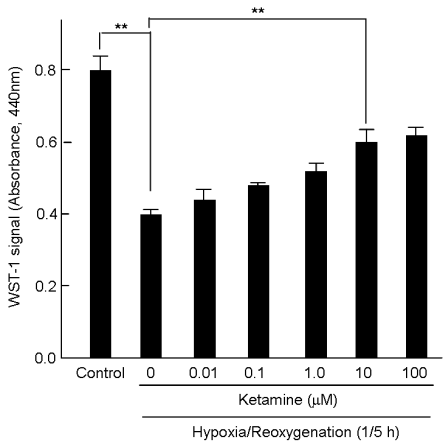

Figure 1. The effect of ketamine on survival of hypoxia-reoxygenated cardiomyocytes. Cardiomyocytes were plated in triplicate wells of 96 well plates at a density of 1 x 10^4 per well, and put in hypoxic chamber for 1 hr prior to exposure to reoxygenation with the above

concentration of ketamine for 5 hr. Cell viability was determined by the MTT assay. WST-1 signal on vertical axis represents the degree of cell survival. (**: p-value < 0.01)

2. The effect of ketamine on activity of proteins related to survival of hypoxia-reoxygenated cardiomyocytes

The phosphorylation of Akt at Ser^{473} and Erk1,2 (42 and 44 kDa) was detected by immunoblot assay. As shown in Figure 2, the phosphorylation activities of both Akt and Erk were obviously decreased in hypoxia-reoxygenated cardiomyocyte compared to the normal cells. The treatment of hypoxia-reoxygenated cells with 10 μM of ketamine resulted in the increase of the activities of Akt and Erk. But phosphorylation activities of both Akt and Erk in hypoxia-reoxygenated cells treated with ketamine was not the same as in normal cells.

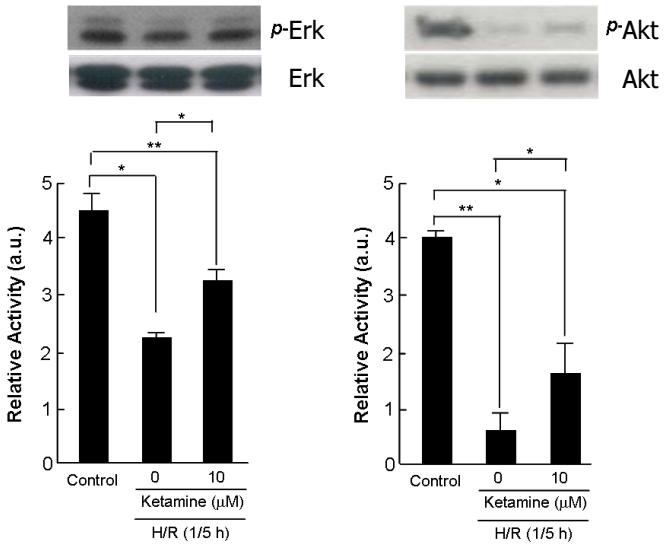

Figure 2. The effect of ketamine on activity of proteins related to survival of hypoxia-reoxygenated cardiomyocytes. Western blot analysis of phosphorylaton of Erk and Akt (*p*-Erk and *p*-Akt) in cardiomyocytes exposed to hypoxic chamber for 1 hr prior to exposure to reoxygenation with 0 or 10 μM ketamine for 5 hr. Each signal was quantified by scanning densitometry and the figure shows the levels of each activity as relative value of the maximal level of *p*-Erk and *p*-Akt. Western blot was repeated three times. (*: p-value < 0.05, ** : p-value < 0.01)

3. The effect of ketamine on activity of proteins related to apoptosis of hypoxia-reoxygenated cardiomyocytes

Hypoxia-reoxygenation induced the increased expression of pro-apoptotic protein, Bax and cytochrome C and the decreased expression of anti-apoptotic protein, Bcl-2 from the normal condition (Figure 3). Following the treatment of hypoxia-reoxygenation cell with 10 μM of ketamine, the expression level of Bax and cytochrome C was decreased and Bcl-2 was increased.

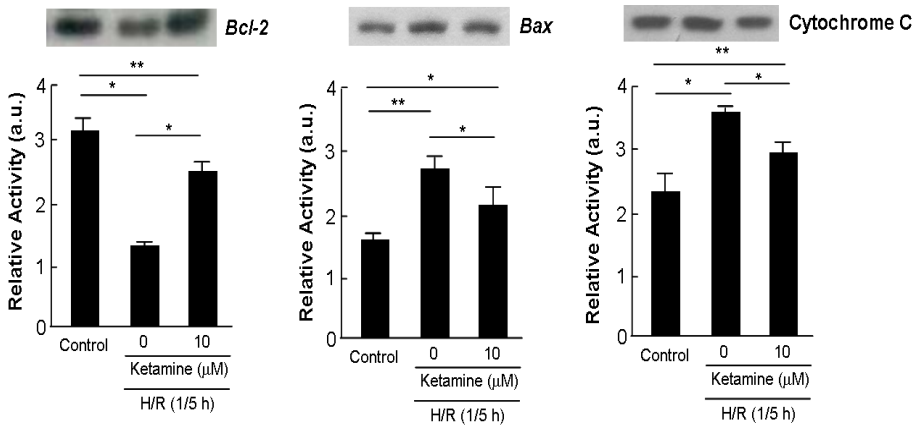

Figure 3. The effect of ketamine on activity of proteins related to apoptosis of hypoxia-reoxygenated cardiomyocytes. Western blot analysis of Bcl-2, Bax, and cytochrome C in cardiomyocytes exposed to hypoxic chamber for 1 hr prior to exposure to reoxygenation with 0 or 10 μM ketamine for 5 hr. Western blot was repeated three times. (*: p-value < 0.05, ** : p-value < 0.01)

4. The effect of ketamine on activity of caspase3 of hypoxia-reoxygenated cardiomyocytes

When the hypoxia-reoxygenation activates the caspase3 in cardiomyocyte, DEVD-*p*NA, a substrate was cleaved vigorously by the enzyme and the increased free *p*NA light absorbance can be quantified at 405nm (Figure 4). But the activity of caspase3 was reduced sharply in the treatment of hypoxia-reoxygenated cardiomyocytes with

10 μM of ketamine due to its protective effect from apoptosis of cardiomyocytes.

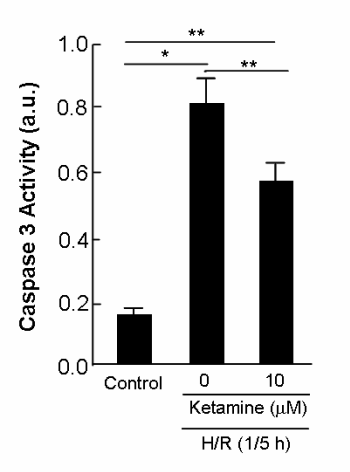

Figure 4. The effect of ketamine on activity of caspase3 of hypoxia-reoxygenated cardiomyocytes. Relative caspase3 activity was determined using ApopTarget™ Capase-3 Colorimetric Protease Assay in cardiomyocytes exposed to hypoxic chamber for 1 hr prior to exposure to reoxygenation with 0 or 10 μM ketamine for 5 hr. Data denote the means ± S.E.M. of 2~3 replicate measurements in three different cell cultures. (*: p-value < 0.05, ** : p-value < 0.01)

5. The effect of ketamine on intracellular calcium concentration of hypoxia-reoxygenated cardiomyocytes

To examine the intracellular Ca^{2+} overload in hypoxia-reoxygenated

cardiomyocytes, the fluorescence intensity method was used in cardiomyocyte loaded with fluo-4 AM. Figure 5 showed that the intracellular calcium level of hypoxia-reoxygenated cells was much higher than that of the normal cells but the treatment of hypoxia-reoxygenated cells with 10 μM of ketamine induced the reduction of intracellular calcium level.

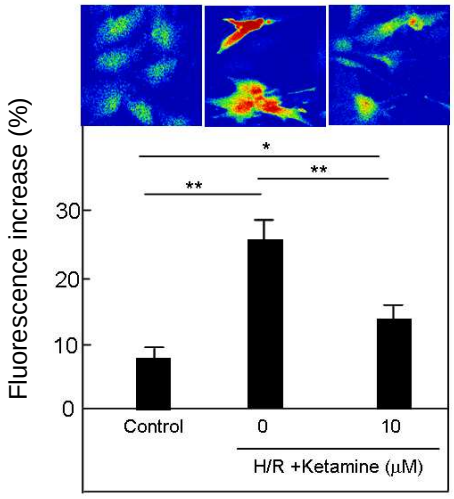

Figure 5. The effect of ketamine on intracellular calcium concentration of hypoxia-reoxygenated cardiomyocytes. Cardiomyocytes were exposed to hypoxic chamber for 1 hr prior to exposure to reoxygenation with 0 or10 μM ketamine for 5 hr. Bright red color in the picture represents high Ca^{2+} concentration in cardiomyocytes. Cytosolic free Ca^{2+} concentration is determined with relative fluorescence intensity. The phrase of 'Fluorescence increase' in vertical axis presents the degree of increase in brightness of most bright area in cellular matrix compared to least bright area. (*: p-value < 0.05, ** : p-value < 0.01)

6. The effect of ketamine on genes coding for Ca^{2+}-binding proteins of hypoxia-reoxygenated cardiomyocytes

The transcript level of calsequestrin in hypoxia-reoxygenated cardiomyocytes was similar to that of normal cells. But the treatment of hypoxia-reoxygenated cells with 10 μM of ketamine increased the expression of calsequestrin (Figure 6).

Compared with normal cardiomyocyte, the expression of calreticulin was significantly decreased in hypoxia-reoxygenated cardiomyocyte but the treatment of hypoxia-reoxygenated cells with 10μM of ketamine induced a large increase in the expression of calreticulin (Figure 6).

As shown in Figure 6, the transcript patterns of calmodulin were totally different from calreticulin. The transcript level of calmodulin in hypoxia-reoxygenated cells was higher than that of the normal cardiomyocyte but was more or less similar in normal cell and hypoxia-reoxygenated cell treated with 10 μM of ketamine.

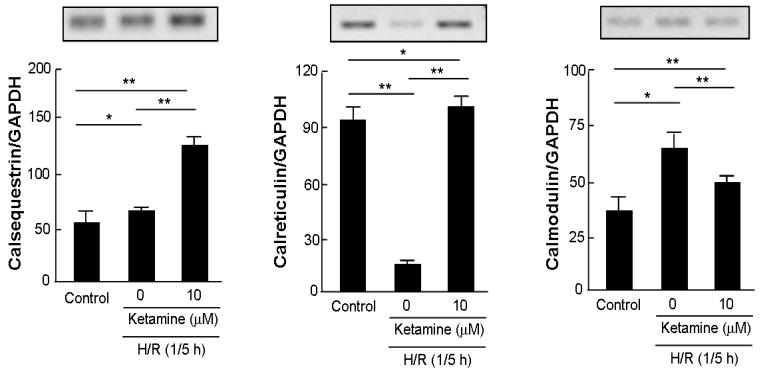

Figure 6. The effect of ketamine on genes coding for Ca^{2+}-handling protein of hypoxia-reoxygenated cardiomyocytes. Cardiomyocytes were exposed to hypoxic chamber for 1 hr prior to exposure to reoxygenation with 0 or 10 μM ketamine for

5 hr. mRNA expression of genes was amplified by RT-PCR and its products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. GAPDH is glyceraldehydes-3-phosphate dehydrogenase. (*: p-value < 0.05, ** : p-value < 0.01)

7. The effect of ketamine on genes coding for ion channels of hypoxia-reoxygenated cardiomyocytes

Figure 7 shows the representative examples of K^+ channel in hypoxia-reoxygenated cardiomyocytes treated with or without 10 μM of ketamine. The expression of mRNA of the inward rectifying K^+ channel (K_{ir}) 3.4, and K_{ir} 6.1 was significantly lower in hypoxia-reoxygenated cardiomyocytes compared with the normal cells. After the treatment of hypoxia-reoxygenated cells with 10 μM of ketamine, the transcript levels of K_{ir} 3.4 and K_{ir} 6.1 were increased. The transcript pattern of L-type Ca^{2+}-channel was as same as K_{ir} 3.4, and K_{ir} 6.1.

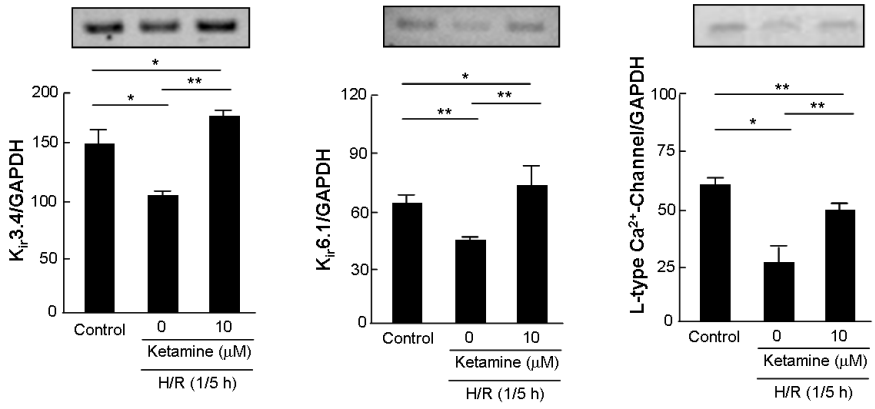

Figure 7. The effect of ketamine on genes coding for ion channels of hypoxia-

reoxygenated cardiomyocytes. Cardiomyocytes were exposed to hypoxic chamber for 1 hr prior to exposure to reoxygenation with 0 or 10 μM ketamine for 5 hr. mRNA expression of genes were amplified by RT-PCR and its products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. GAPDH is glyceraldehydes-3-phosphate dehydrogenase and K_{ir} is inward rectifying K^+ channel. (*: p-value < 0.05, ** : p-value < 0.01)

8. The effect of ketamine on genes coding for ion exchangers of hypoxia-reoxygenated cardiomyocytes

The expressions of genes coding ion exchangers were different from each other in hypoxia-reoxygenated cardiomyocytes treated with or without 10 μM of ketamine.

In sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase (SERCA) 2a, the gene expression of hypoxia-reoxygenated cardiomyocytes treated with 10 μM of ketamine was much more decreased than that of hypoxia-reoxygenated cells without ketamine and it was as same as normal cells (Figure 8).

Plasma membrane Ca^{2+}-adenosine triphosphatase (PMCA) 1 formed a distinct contrast to SERCA2a in the transcript pattern. The treatment of hypoxia-reoxygenated cardiomyocytes with 10 μM of ketamine induced the increase of expression of gene coding PMCA1 compared with hypoxia-reoxygenated cells without ketamine.

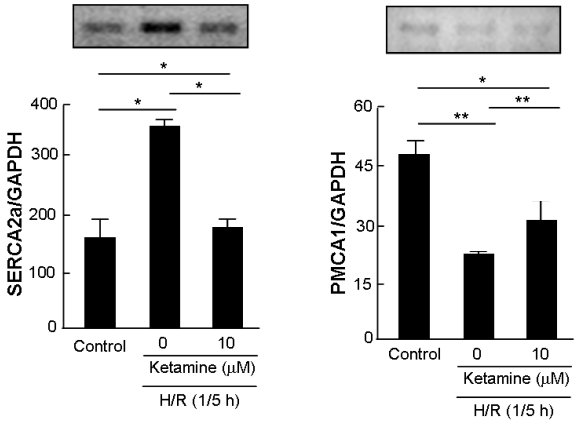

Figure 8. The effect of ketamine on genes coding for ion exchangers of hypoxia-reoxygenated cardiomyocytes. Cardiomyocytes were exposed to hypoxic chamber for 1 hr prior to exposure to reoxygenation with 0 or 10 μM ketamine for 5 hr. mRNA expression of genes were amplified by RT-PCR and its products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. GAPDH is glyceraldehydes-3-phosphate dehydrogenase. SERCA is sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase and PMCA is plasma membrane Ca^{2+}-adenosine triphosphatase. (*: p-value < 0.05, ** : p-value < 0.01)

IV. DISCUSSION

The myocardial protection by intravenous anesthetics (morphine and propofol) and volatile anesthetics (isoflurane and sevoflurane) has been relatively well investigated in the previous studies.[14,15] It has been known that these agents reduce the myocardial damage caused by hypoxia-reoxygenation. Other anesthetics used in the clinical practice, such as fentanyl, ketamine, barbiturates and benzodiazepines have been much less studied, and their abilities as cardioprotectors are currently unknown. Some studies indicate that different anesthetics have different mechanisms of myocardial protection.

In general, myocardial ischemia initiates a range of cellular events, which are initially mild and become progressively damaging with the increased period of ischemia. Although the reperfusion is the termination of ischemia and an essential condition for the cellular survival and restoration of normal function, it paradoxically causes damage to the cell.[16] Ischemia reduces the adequate oxygen supply, which results in the depletion of adenosine triphosphate (ATP). This inhibits ATP-driven Na^+-K^+ pumps, increasing $[Na^+]_i$. $[H^+]_i$ is also increased by the poor washout of metabolites and the inhibition of mitochondrial oxidation of nicotinamide-adenine dinucleotide (NADH). Increased $[H^+]_i$ enhances Na^+-H^+ exchange to retain normal pH_i, leading to the increased $[Na^+]_i$, hence $[Ca^{2+}]_i$ is augmented via Na^+-Ca^{2+} exchange.[17] High $[Ca^{2+}]_i$ degrades proteins and phospholipids. Ischemia also induces

the production of free radical and the major targets of free radicals are lipid and protein, which are the components of structure in plasma membrane and enzyme. On reperfusion, $[H^+]_i$ outside the cell is abruptly reduced to a normal level because it is washed out. This results in an increase of $[Ca^{2+}]_i$ due to the enhanced Na^+-H^+ and Na^+-Ca^{2+} exchange.[18] Reperfusion also results in a burst of free radical generation because oxygen is abundantly supplied.[19] Ca^{2+} and free radicals injure the heart further at reperfusion.

In this study, it was found that the treatment of hypoxia-reoxygenated cardiomyocytes with ketamine improved the cellular survival (Figure 1). Hypoxia-reoxygenation decreased obviously the survival of cardiomyocytes. The cellular survival was increased with the increase in the concentration of ketamine and there was no difference in the survival between in 10 μM and 100 μM ketamine. In human the plasma concentration of ketamine is 9.3 ± 0.8 μM by the intravenous injection of 2 mg/kg as loading dose accompanied by the continuous intravenous infusion for maintenance of anesthesia,[20] so the positive effect of ketamine on the survival of hypoxia-reoxygenated cardiomyocytes can be clinically meaningful.

In the mechanisms of cellular survival and proliferation, the activation of Erk1,2 plays an important role in the gene regulation and PI3-kinase/Akt signaling pathway is also pro-survival and anti-apoptotic signal.[21] Akt is phosphorylated at two sites due to the activation of enzyme activity: Thr^{308} in the catalytic domain and Ser^{473} in the cytoplasmic domain.[22] Erk is one of dual specificity kinases in mitogen-activated

protein kinases (MAPKs).[23] In cardiomyocyte, the members of the Erk MAPK family have been implicated in survival signaling in response to hypoxia-reoxygenation, oxidative stress, and β-adrenergic stimulation.[24-26] In many systems, activations of Akt and Erks provide potent pro-survival signal. Under pathological condition of hypoxia-reoxygenation, enhanced survival of neonatal rat ventricular cardiomyocyte seems to be related to increased activation of Akt and Erk (Figure 2).

It has been known that hypoxia-reoxygenation is one of apoptotic stimulus in cardiomyocyte.[27] Apoptosis through the mitochondrial pathway is partly regulated by the interaction of Bcl-2 family proteins.[28] These proteins may be anti-apoptotic (Bcl-2) or pro-apoptotic (Bax) and pro-apoptotic protein induces conformational change which triggers the release of cytochrome C from the mitochondrial membrane space into the cytosol.[29] In relation to pro-apoptosis via hypoxia-reoxygenation, this study suggested that the treatment of hypoxia-reoxygenated cardiomyocyte with 10 μM ketamine led to the reduction of apoptosis. Caspases are activated following cleavage at specific aspartate cleavage sites in response to apoptotic stimulus and caspase3 activation has been documented in the myocardium of end-stage heart failure patients.[30-32] Generally, ischemia causes mitochondrial permeability transition (MPT)-related cytochrome C release, which then induces caspase3 activation and apoptosis.[24,28] Ketamine attenuated the expression and activity of pro-apoptotic signal such as Bax, cytochrome C, and caspase3 but enhanced the expression of Bcl-2 in this study (Figure 3, 4). Therefore it can be supposed that ketamine increases the cellular

survival of hypoxia-reoxygenated cardiomyocytes by the interference in the expression of pro-apoptotic proteins.

In this study there was an obvious increase of intracellular Ca^{2+} level in hypoxia-reoxygenated cardiomyocytes compared to the normal cells and ketamine inhibited the increase of intracellular Ca^{2+} level at the concentration of 10 μM (Figure 5). The increased level of intracellular calcium can activate signaling pathway leading to the apoptosis and cell death caused by calcium overloading has been implicated in myocardium injury of hypoxia-reoxygenation. Ketamine could not reduce the intracellular Ca^{2+} level to normal in this study. But considering that the intracellular Ca^{2+} level of hypoxia-reoxygenated cardiomyocytes was decreased and its cellular survival increased by ketamine, it is possible to suppose that the positive effect of ketamine on cellular survival in hypoxia-reoxygenation is related to its ability to reduce intracellular Ca^{2+} level.

Ca^{2+} does not only have the role as a second messenger but it is also the effector of the contractile event.[33] Therefore, several proteins are involved to ensure a finely tuned Ca^{2+} mobilization in the cardiomyocyte, including Ca^{2+}-binding proteins, ion channels and ion pumps.

Calsequestrin is a major Ca^{2+}-binding protein in the sarcoplasmic reticulum of cardiomyocyte.[34] It has two major roles, one is Ca^{2+} buffering by the Ca^{2+} -binding properties and the other is the regulation of the activity of Ca^{2+} release channel by interaction with ryanodine receptor (RyR). In case of calsequestrin overexpression it

inhibits so strongly RyR channel by binding directly to the RyR, which reduces intracellular Ca^{2+} release.[35,36] In this study the expression of gene coding calsequestrin was largely increased by ketamine in the hypoxia-reoxygenated cardiomyocytes (Figure 6). It can be suggested that ketamine inhibited the intracellular Ca^{2+} increase in hypoxia-reoxygenated cardiomyocytes by means of calsequestrin overexpression.

Calreticulin is a Ca^{2+} -binding chaperone of the sarcoplasmic reticulum (SR).[37] Overexpression of calreticulin leads to the prevention of oxidant-induced intracellular Ca^{2+} increase and results in cytoprotection.[38,39] In this study the expression of gene coding calreticulin was excessively decreased in hypoxia-reoxygenated cardiomyocytes (Figure 6). However the administration of ketamine in hypoxia-reoxygenated cardiomyocytes restored the expression of the gene to nearly normal. This result represents that the effect of ketamine on calreticulin in hypoxia-reoxygenated cardiomyocytes contributed to the reduction of elevated intracellular Ca^{2+} level.

Calmodulin is a typical Ca^{2+}-binding protein and a important second messenger in intracellular signal transduction.[40] It is involved in many important pathways of intracellular signal transduction directly or indirectly.[33] So it is hard to expect specific cellular event by the expression of calmodulin alone. But the expression of gene coding calmodulin was increased in the hypoxia-reoxygenated cardiomyocytes and the administration of ketamine lowered the increase of expression (Figure 6). A further study is necessary to define the relationship between the expression of gene

coding calmodulin and the intracellular Ca^{2+} level.

In cardiomyocyte the excitation-contraction process is triggered by a depolarization, which induces the opening of specialized Ca^{2+}channel, L-type Ca^{2+}- channel.[41] The activation of L-type Ca^{2+}- channel allows a small amount of Ca^{2+} to enter the cytosol. The Ca^{2+} entering the cytosol can reach RyR, which produces a massive release of Ca^{2+} from the SR into the cytosol. In this study the expression of gene coding L-type Ca^{2+}- channel was decreased in the hypoxia-reoxygenated cardiomyocytes and ketamine diminished the decrease of the gene expression (Figure 7). According to this result it is not certain whether L-type Ca^{2+}- channel played a major role in the change of intracellular Ca^{2+} level in hypoxia-reoxygenated cardiomyocytes. But ketamine seems to make the expression of gene coding L-type Ca^{2+}- channel nearly normal in hypoxia-reoxygenation.

Gene expression of ATP-sensitive K^+ (K_{ATP}) channel mediates its cardioprotective effect and increased expression of K_{ir} 6.1 is related to myocardial protection.[42-45] In this study ketamine enhanced the expression of gene coding K_{ir} 6.1 and the cell survival in hypoxia-reoxygenation, so it seems that ketamine has a protective effect in hypoxia-reoxygenated cardiomyocyte via increased expression of K_{ir} 6.1 (Figure 7). K_{ir} 3.4 is another K_{ATP} channel but there are still numerous opposed studies for expression.[42] In this study, the pattern of K_{ir} 3.4 was as same as K_{ir} 6.1 (Figure 7).

Sarcoplasmic reticulum Ca^{2+}- ATPase (SERCA) 2a is the most abundant isoform of the Ca^{2+}-ATPase within the heart.[41] It is a conerstone molecules for maintaining a

balanced concentration of Ca^{2+} during the cardiac cycle, since it controls the transport of Ca^{2+} to SR during relaxation.[46] Many studies showed reduced expression of SERCA2a but some reports provided unaltered expression of SERCA2a in failing hearts.[12,47-49] In this study the expression of gene coding SERCA2a was largely increased in hypoxia-reoxygenated cardiomyocytes and it returned to nearly normal level by ketamine (Figure 8). Considering the role of SERCA2a to transport Ca^{2+} to SR, it is difficult to explain this discordance between the elevated intracellular Ca^{2+} level and the increased gene expression of SERCA2a. But it is also hard to elucidate the change of intracellular Ca^{2+} level by the gene expression of SERCA2a alone because SERCA2a is specially regulated by phospholamban (PLN) and other molecules are also involved in regulating the interaction between SERCA2a and PLN, i.e., protein phosphatase I and inhibitor I.[41,50] Although hypoxia-reoxygenation led to the increased expression of SERCA2a in this study , it demonstrated that ketamine was the cardioprotective agent because the expression level of SERCA2a in hypoxia-reoxygenated cardiomyocyte was as same as normal cell by the administration of ketamine.

Plasma membrane Ca^{2+}- ATPase (PMCA) transport Ca^{2+} out of the cell by using the energy stored in ATP.[46,51] Overexpression of PMCA leads to a reduction in the activity of the SERCA. Conversely the expression of the SERCA has a direct influence on the activity of PMCA. This may indicate that the gene expressions of the PMCA and SERCA are interdependent on each other.[51] Hypoxia-reoxygenation has been known

to reduce the expression of PMCA1 in brain and cardiomyocyte.[52] In this study the expression of gene coding PMCA1 was decreased in hypoxia-reoxygenated cardiomyocytes as expected and ketamine diminished the reduction of gene expression (Figure 8).

There are two conflicting opinions about the cardioprotective effect of ketamine. One is that ketamine has a weak scavenging effect and a weak effect to suppress reactive oxygen species (ROS) production by neutrophils.[53,54] The other is that ketamine blocks the cardioprotective effect of ischemic preconditioning in vivo.[55] So it is not simple to conclude whether ketamine has a cardioprotective effect but this study can make a suggestion that ketamine protects cardiomyocytes in hypoxia-reoxygenation. All ion channels and ion pumps regulating intracellular Ca^{2+} level are not investigated in this study. Especially, experiments for major proteins regulating intracellular Ca^{2+} level, RyR channel and Na^{+}- Ca^{2+} exchanger, were not performed. It is also difficult to explain the change of intracellular Ca^{2+} level without the investigation into the factors regulating the activities of these channels and pumps. These are limitations of this study, so detailed study should be followed.

V. CONCLUSION

In the present study it was performed to investigate the changes in survival proteins, apoptosis-related proteins and Ca^{2+} overload in hypoxia-reoxygenated cardiomyocytes. It was also performed to compare experimental findings with results from gene expression of Ca^{2+}-binding proteins and various ion channels as well as ion exchangers by RT-PCR.

The results were as follows :

1. The survival rate of hypoxia-reoxygenated cardiomyocytes treated with ketamine was more increased than without ketamine.
2. Treatment of hypoxia-reoxygenated cardiomyocytes with 10 μM ketamine resulted in the increase of the activities of Akt and Erk than that of the cells without ketamine.
3. Ketamine decreased the expression level of Bax and cytochrome C and the activity of caspase3 but increased the expression of Bcl-2 in hypoxia-reoxygenated cardiomyocytes.
4. The treatment of hypoxia-reoxygenated cardiomyocytes with 10 μM ketamine induced the reduction of intracellular calcium level.
5. The expressions of calsequestrin and calreticulin were increased by the treatment with 10 μM ketamine but not in calmodulin.
6. The expressions of K_{ir} 3.4, K_{ir} 6.1 and L-type Ca^{2+}-channel were increased by

the treatment with 10 μM ketamine.

7. The expression of SERCA2a was decreased by the treatment 10 μM ketamine, while that of PMCA1 increased.

Ketamine increases the survival of cardiomyocyte in hypoxia-reoxygenation, and this protective effect seems to be related to the change in the expression of the proteins regulating the intracellular calcium level. Therefore ketamine may be beneficial to the myocardial protection from hypoxic injury and a further clinical research into ketamine and its protective effect should be performed for practical application.

REFERENCES

1. White PF, Way WL, Trevor AJ. Ketamine: Its pharmacology and therapeutic uses. Anesthesiology 1982;56:119-36.
2. Green SM, Johnson NE. Ketamine sedation for pediatric procedures: Part 2. Review and implications. Ann Emerg Med 1990;19:1033-46.
3. Shimoyama M, Shimoyama N, Gorman AL, Elliot KJ, Inturrisi CE. Oral ketamine is antinociceptive in the rat formalin test: role of the metabolite, norketamine. Pain 1999;81:85-93.
4. Yanagihara Y, Kariya S, Ohtani M, Uchino K, Aoyama T, Yamamura Y, et al. Involvement of CYP2B6 in N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2001;29:887-90.
5. Hijazi Y, Boulieu R. Contribution of CYP3A4, CYP2B6 and CYP2C9 isoforms to N-demethylation of ketamine in human liver microsomes. Drug Metab Dispos 2002;30:853-8.
6. Weber M, Motin L, Gaul S, Beker F, Fink RH, Adams DJ. Intravenous anaesthetics inhibit nicotinic acetylcholine receptor-mediated currents and Ca^{2+} transients in rat intracardiac ganglion neurons. Br J Pharmacol 2005;144:98-107.
7. Ko SH, Yu CW, Lee SK, Choe H, Chung MJ, Kwak YG, et al. Propofol attenuates ischemia-reperfusion injury in the isolated rat heart. Anesth Analg 1997;85:719-24.

8. Kato R, Foex P. Myocardial protection by anesthetic agents against ischemia-reperfusion injury:an update for anesthesiologists. Can J Anaesth 2002;49:777-91.
9. Liang BT, Gross GJ. Direct preconditioning of cardiac myocytes via opioid receptors and K_{ATP} channels. Circ Res 1999;84:1396-400.
10. Schultz JE, Hsu AK, Gross GJ. Ischemic preconditioning in the intact rat heart is mediated by δ1- but not μ- or κ-opioid receptors. Circulation 1998;97:1282-9.
11. Heindl B, Reichle FM, Zahler S, Conzen PF, Becker BF. Sevoflurane and isoflurane protect the reperfused guinea pig heart by reducing postischemic adhesion of polymorphonuclear neutrophils. Anesthesiology 1999;91:521-30.
12. Huke S, Liu LH, Biniakiewicz D, Abraham WT, Periasamv M. Altered force-frequency response in non-failing hearts with decreased SERCA pump-level. Cardiovasc Res 2003;59:668-77.
13. Terracciano CM, Hardy J, Birks EJ, Khaghani A, Banner NR, Yacoub MH. Clinical recovery from end -stage heart failure using left-ventricular assist device and pharmacological theraphy correlates with increased sarcoplasmic reticulum calcium content but not with regression of cellular hypertrophy. Circulation 2004;109:2263-5.
14. Ismaeil MS, Tkachenko I, Gamperl AK, Hickey RF, Cason BA. Mechanisms of isoflurane-induced myocardial preconditioning in rabbits. Anesthesiology 1999 ;90:812-21.

15. De Hert SG, Turani F, Mathur S, Stowe DF. Cardioprotection with volatile anesthetics: mechanisms and clinical implications. Anesth Analg. 2005;100:1584-93.

16. Maxwell SRJ, Lip GYH. Reperfusion injury: a review of the pathophysiology, clinical manifestations and therapeutic options. Int J Cardiol 1997; 58: 95−117.

17. Clanachan AS. Contribution of protons to post-ischemic Na^+ and Ca^{2+} overload and left ventricular mechanical dysfunction. J Cardiovasc Electrophysiol 2006;17 Suppl 1:S141-S148.

18. Stromer H, de Groot MC, Horn M, Faul C, Leupold A, Morgan JP, et al. Na^+/H^+ exchange inhibition with HOE642 improves postischemic recovery due to attenuation of Ca^{2+} overload and prolonged acidosis on reperfusion. Circulation 2000;101:2749-55.

19. Jordan JE, Zhao ZQ, Vinten-Johansen J. The role of neutrophils in myocardial ischemia–reperfusion injury. Cardiovasc Res 1999; 43: 860–78.

20. Idvall J, Ahlgren I, Aronsen KR, Stenberg P. Ketamine infusions: pharmacokinetics and clinical effects. Br J Anaesth 1979;51:1167-73.

21. Amaravadi R, Thompson CB. The survival kinases Akt and Pim as potential pharmacological targets. J Clin Invest 2005;115:2618-24.

22. Partovian C, Simons M. Regulation of protein kinase B/Akt activity and Ser^{473} phosphorylation by protein kinase Cα in endothelial cells. Cell Signal 2004;16:951-7.

23. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev 2004;68:320-44.

24. Krijnen PA, Nijmeijer R, Meijer CJ, Visser CA, Hack CE, Niessen HW. Apoptosis in myocardial ischaemia and infarction. J Clin Pathol 2002;55:801-11.

25. Sheng Z, Knowlton K, Chen J, Hoshijima M, Brown JH, Chien KR. Cardiotrophin-1(CT-1) inhibition of cardiac myocyte apoptosis via a mitogen-activated protein kinase-dependent pathway. Divergence from downstream CT-1 signals for myocardial cell hypertrophy. J Biol Chem 1997;272:5783-91.

26. De Windt LJ, Lim HW, Taigen T, Wencker D, Condorelli G, Dorn 2nd GW, et al. Calcineurin-mediated hypertrophy protects cardiomyocytes from apoptosis in vitro and in vivo: an apoptosis independent medel of dilated heart failure. Cir Res 2000;86:255.

27. Bennett MR. Apoptosis in the cardiovascular system. Heart 2002;87:480-7.

28. Bouchier-Hayes L, Lartigue L, Newmeyer DD. Mitochondria: pharmacological manipulation of cell death. J Clin Invest 2005;115:2640-7.

29. Sjöström J, Bergh J. How apoptosis is regulated, and what goes wrong in cancer. BMJ 2001;322:1538-9.

30. Lavrik IN, Golks A, Krammer PH. Caspases: pharmacological manipulation of cell death. J Clin Invest 2005;115:2665-72.

31. Guicciardi ME, Gores GJ. Apoptosis: a mechanism of acute and chronic liver

injury. Gut 2005;54:1024-33.

32. Kumar D, Lou H, Singal PK. Oxidative stress and apoptosis in heart dysfunction. Herz 2002;27:662-8.

33. Maier LS, Bers DM. Calcium, calmodulin, and calcium-calmodulin kinase II: heartbeat to heartbeat and beyond. J Mol Cell Cardiol 2002;34:919-39.

34. Beard NA, Laver DR, Dulhunty AF. Calsequestrin and the calcium release channel of skeletal and cardiac muscle. Prog Biophys Mol Biol 2004;85:33-69.

35. Schmidt AG, Kadambi VJ, Ball N, Sato Y, Walsh RA, Kranias EG, et al. Cardiac-specific overexpression of calsequestrin results in left ventricular hypertrophy, depressed force-frequency relation and pulsus alternans in vivo. J Mol Cell Cardiol 2000;32:1735-44.

36. Sato Y, Freguson DG, Sako H, Dorn GW, Kadambi VJ, Yatani A, et al. Cardiac-specific overexpression of mouse cardiac calsequestrin is associated with depressed cardiovascular function and hypertrophy in transgenic mice. J Biol Chem 1998;273:28470-7.

37. Bedard K, Szabo E, Michalak M, Opas M. Cellular functions of endoplasmic reticulum chaperones calreticulin, calnexin, and ERp57. Int Rev Cytol 2005;245:91-121.

38. Liu H, Bowes RC, van de Water B, Sillence C, Nagelkerke JF, Stevens JL. Endoplasmic reticulum chaperones GRP78 and calreticulin prevent oxidative stress Ca^{2+} disturbances and cell death in renal epithelial cells. J Biol Chem 1997;272:21751-9.

39. Liu H, Miller E, van de Water B, Stevens JL. Endoplasmic reticulum stress protein stress induction through targeted ribozyme driven by a stress-inducible promoter. J Biol Chem 1998;273:12858-72.

40. Ganong WF. Review of medical physiology. 20th ed. New York: Lange medical books/McGraw Hill; 2001.

41. Gianni D, Chan J, Gwathmey JK, del Monte F, Hajjar RJ. SERCA2a in heart failure: role and therapeutic prospects. J Bioenerg Biomembr 2005;37:375-80.

42. Lawrence KM, Chanalaris A, Scarabelli T, Hubank M, Pasini E, Townsend PA, et al. KATP channel gene expression is induced by urocortin and mediates its cardioprotective effect. Circulaion 2002;106:1556-62.

43. Bell SP, Sack MN, Patel A, Opie LH, Yellon DM. Delta opioid receptor stimulation mimics ischemic preconditioning in human heart muscle. J Am Coll Cardiol 2000;36:2296-302.

44. Zaugg M, Schaub MC. Signaling and cellular mechanisms in cardiac protection by ischemic and pharmacological preconditioning. J Muscle Res Cell Motil 2003;24:219-49.

45. Fujita A, Kurachi Y. Molecular aspects of ATP-sensitive K^+ channels in the cardiovascular system and K^+ channel openers. Pharmacol Ther 2000 ;85:39-53.

46. Nelson DL, Cox MM, Lehninger AL. Lehninger principle of biochemistry. 4th ed. New York: W.H. Freeman; 2005.

47. Chaudhary KW, Rossman EI, Piaccentino V 3rd, Kenessev A, Weber C, Gaughan

JP, et al. Altered myocardial Ca^{2+} cycling after left ventricular assist device support in the failing human heart. J Am Coll Cardiol 2004;44:837-45.

48. Borlak J, Thum T. Hallmarks of ion channel gene expression in end-stage heart failure. FASEB J 2003;17:1592-1608.

49. Houser SR, Piacentino VIII, Weisser J. Abnormalities of calcium cycling in the hypertrophied and failing heart. J Mol Cell Cardiol 2000;32:1595-1607.

50. Mirit E, Gross C, Hasin Y, Palmon A, Horowitz M. Changes in cardiac mechanics with heat acclimation: Adrenergic signaling and SR-Ca regulatory proteins. Am J Physiol Regul Integr Comp Physiol 2000;279:R77-85.

51. Guerini D. The significance of the isoforms of plasma membrane calcium ATPase. Cell Tissue Res 1998;292:191-7.

52. Lehotsky J, Kaplan P, Racay P, Mezesova B, Raeymaekers L. Distribution of plasma membrane Ca^{2+} pump (PMCA) isofroms in the gerbil brain: effect of ischemia-reperfusion injury. Neurochemistry International 1999;35:221-7.

53. Weigand MA, Schmidt H, Zhao Q, Plaschke K, Martin E, Bardenheuer HJ. Ketamine modulates the stimulated adhesion molecule expression on human neutrophils in vitro. Anesth Analg 2000;90:206-12.

54. Kevin LG, Novalija E, Stowe DF. Reactive oxygen species as mediators of cardiac injury and protection: the relevance to anesthesia practice. Anesth Analg 2005;101:1275-87.

55. Müllenheim J, Fräβdorf J, Preckel B, Thämer V, Schlack W. Ketamine, but not

S(+)-ketamine, blocks ischemic preconditioning in rabbit hearts in vivo. Anesthesiology 2001;94:630-6.

ABSTRACT (IN KOREAN)

Ketamine이 투여된 심근세포의 칼슘 항상성 관련 단백질들의 발현과 보호효과

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노 장 호

Ketamine은 다른종류의 정맥마취제나 흡입마취제보다 안정적인 혈역학적인 특징을 가지고 있으므로 임상적으로 위독한 환자에게 마취를 유도할 때 유용하게 사용된다. 마취약제가 심근세포의 칼슘 항상성에 미치는 영향에 대하여 진행된 연구는 거의 드물며 심근세포의 허혈-재관류 손상에 대한 ketamine의 보호효과 또한 잘 알려져 있지 않다

본 연구에서는 ketamine이 허혈-재관류 처리된 심근세포의 생존에 미치는 영향을 알아보고 세포의 사멸에 관련된 단백질들의 발현을 조사하였다. 또한 허혈-재관류 처리된 심근세포에서 ketamine이 세포내 칼슘농도의 변화와 칼슘 항상성 관련 단백질들의 발현에 미치는 영향에 대하여 알아보았다.

백서의 심근세포를 분리배양하여 세포의 생존률을 조사하기 위하여 MTT assay를 실시하고 세포내 칼슘농도의 변화를 측정하기 위하여 Confocal microscopy와 형광측정법을 이용하였다. 또한 역전사 중합효소연쇄반응을 실시하여 세포의 사멸에 관련된 단백질들의 발현과 칼슘 항상성 관련 단백질들의 발현을 조사하였다. 실험을 진행한 결과, 허혈에 따른 재관류시 심근세포에 0~100 μM의 ketamine을 각각 투여함에 따라 세포의 생존률이 다소 증가함을 확인하였다. 또한 재관류시 심근세포에 10 μM의 ketamine을 투여할 경우 세포의 생존에 관련된 단백질인 Erk와 Akt의 발현은 증가하며 세포의 사멸에 관계되는 Bax과 cytochrome C의 발현은 억제되는 것을 관찰할 수 있었다. 세포내 칼슘농도의 경우 허혈-재관류에 의해 증가하였으나 재관류시 심근세포에 10 μM의 ketamine 을 투여할 경우 칼슘농도의 증가는 상당히 억제되었다. 칼슘 항상성 관련 단백질 중 calsequestrin과 calreticulin, 이온 채널들, PMCA1의 발현은 ketamine의 투여로 증가하였다.

결론적으로 ketamine은 허혈-재관류 손상에 의한 심근세포의 사멸을 감소시키며 이 심근세포 보호효과는 세포내 칼슘 항상성 관련 단백질들의 발현 변화와 연관된 것으로 추측할 수 있다. 따라서 ketamine은 심근허혈로부터 심근세포를 보호하는 효과를 가지고 있는 것으로 보여지므로 앞으로 이에 대해 많은 연구가 필요할 것으로 생각한다.

핵심되는 말 : 심근보호, 심근세포, 칼슘 항상성, ketamine