

Remifentanil Protects Myocardium through Activation of Anti-Apoptotic Pathways of Survival in Ischemia-Reperfused Rat Heart

H. S. KIM^{1,2}, J. E. CHO³, S. W. HONG⁴, S. O. KIM⁴, J. K. SHIM^{2,3}, Y. L. KWAK^{2,3}

¹Brain Korea 21 Project for Medical Science, ²Anesthesia and Pain Research Institute and

³Department of Anesthesiology and Pain Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea, ⁴Department of Anesthesiology and Pain Medicine, Kyungpook National University, Daegu, Republic of Korea

Received February 23, 2009

Accepted July 31, 2009

On-line August 12, 2009

Summary

Remifentanil is a commonly used opioid in anesthesia with cardioprotective effect in ischemia-reperfused (I/R) heart. We evaluated the influence of remifentanil on myocardial infarct size and expressions of proteins involved in apoptosis in I/R rat heart following various time protocols of remifentanil administration. Artificially ventilated anesthetized Sprague-Dawley rats were subjected to a 30 min of left anterior descending coronary artery occlusion followed by 2 h of reperfusion. Rats were randomly assigned to one of five groups; Sham, I/R only, remifentanil preconditioning, postconditioning and continuous infusion group. Myocardial infarct size, the phosphorylation of ERK1/2, Bcl2, Bax and cytochrome c and the expression of genes influencing Ca²⁺ homeostasis were assessed. In remifentanil-administered rat hearts, regardless of the timing and duration of administration, infarct size was consistently reduced compared to I/R only rats. Remifentanil improved expression of ERK 1/2 and anti-apoptotic protein Bcl2, and expression of sarcoplasmic reticulum genes which were significantly reduced in the I/R rats only. Remifentanil reduced expression of pro-apoptotic protein, Bax and cytochrome c. These suggested that remifentanil produced cardioprotective effect by preserving the expression of proteins involved in anti-apoptotic pathways, and the expression of sarcoplasmic reticulum genes in I/R rat heart, regardless of the timing of administration.

Key words

Anti-apoptosis • Cardioprotection • Ischemia/ reperfusion injury • Remifentanil

Corresponding author

Y. L. Kwak, Department of Anesthesiology and Pain Medicine, Anesthesia and Pain Research Institute, Yonsei University College of Medicine, 134 Shinchon-Dong, Seodaemun-Ku, Seoul 120-752, Republic of Korea. Fax: +82 2 364 2951. E-mail: ylkwak@yuhs.ac

Introduction

Despite restoration of coronary blood flow, ischemia-reperfusion (I/R) injury in myocardium triggers stress signaling processes that eventually result in various degree of myocardial damage (Anaya-Prado *et al.* 2002, Fliss and Gattinger 1996). In attempts to lessen the I/R injury, ischemic preconditioning was introduced as an effective treatment modality (Murry *et al.* 1986). Certain anesthetic agents also possess cardioprotective effects against I/R injury, mimicking ischemic preconditioning, termed pharmacological preconditioning (Schultz and Gross 2001, Tanaka *et al.* 2004). Remifentanil, is an ultra-short-acting, selective, μ -opioid receptor agonist, gaining increasing popularity in cardiac anesthesia (Glass 1995, Patel and Spencer 1996). Like several anesthetics, remifentanil was reported to protect myocardium against I/R injury. In the light of remifentanil's preconditioning effect, the activation of opioid receptors (Peart *et al.* 2005, Yu *et al.* 2007, Zhang *et al.* 2004), protein kinase C (PKC) and mitochondrial adenosine triphosphate-sensitive potassium (K_{ATP}) channels were reported to be important mediators of its cardioprotective action (Zhang *et al.* 2005), although those were observed at doses much

higher than doses used in clinical practice.

Apoptosis, a programmed cell death, contributes significantly to post-ischemic cardiomyocyte death, (Gottlieb and Engler 1999) but evidence is lacking regarding the effect of remifentanil on anti-apoptotic pathways of survival in I/R injury. In mammalian cells, among three major mitogen-activated protein kinases (MAPKs) signaling pathways, activation of extracellular signal related protein kinases (ERK1/2) exerts beneficial effect on post-ischemic myocardial apoptosis (Yue *et al.* 2000).

Thus, we evaluated the effects of the minimal concentration of remifentanil (Yu *et al.* 2007, Zhang *et al.* 2005) against I/R injury on myocardial infarction in rat in terms of the expression of proteins involved in apoptosis signaling cascades including ERK1/2 following various time protocols of remifentanil administration in rat heart. In addition, since the proteins involved in apoptosis significantly affect Ca^{2+} homeostasis, we concomitantly measured expressions of genes associated with Ca^{2+} homeostasis.

Methods

Animals

Male Sprague-Dawley rats (250-300 g) were anesthetized with a single i.p. injection of pentobarbital 60 mg/kg (Yu *et al.* 2007, Zhang *et al.* 2004). Anesthesia was maintained by repeated doses of pentobarbital 25 mg kg^{-1} every 60-90 min. After tracheal intubation, the rats were artificially ventilated (Harvard Apparatus 683, USA) at a rate of 30-35 cycles per minute with oxygen 100 % and a tidal volume of approximately 5 ml. The respiratory rate was varied to maintain the end-tidal CO_2 by 30-35 mm Hg using a capnograph (Datec, Normocap[®], Finland).

The right femoral artery was used for monitoring of mean arterial pressure, and the heart rate was monitored *via* subcutaneous stainless steel electrodes. These were connected to a PowerLab monitoring system (ML845 PowerLab with ML132; AD Instruments, USA). The left femoral vein was cannulated to infuse remifentanil or saline. Rectal temperature was maintained at 38 °C during the entire experiment. The animal experimental procedures were approved by the committee for the Care and Use of Laboratory Animals of our hospital and were performed in accordance with the Committee's Guidelines and Regulations for Animal Care.

Experimental protocol

After a left thoracotomy and pericardiotomy, the heart was exposed and a suture was passed around the left anterior descending (LAD) coronary artery by inserting a small curved Prolene 6.0 needle into the margin of the pulmonary cone, exiting through the middle of a line linking the cone to the atrium. The suture ends were threaded through a small vinyl tube to prepare a snare. After surgical preparation, the rat was allowed to stabilize for 20 min. In all groups, the LAD coronary artery was occluded for 30 min by tightening the snare. Myocardial ischemia was confirmed by the appearance of a regional cyanosis on the epicardium distal to the snare and akinesia or bulging in this area. After 30 min of ischemia, the snare was released and reperfusion allowed for a period of 2 h. Hemodynamic parameters were measured at following time points; 1) at the end of stabilization before ischemia (baseline), 2) at the end of ischemia (or no ischemia), and 3) after 2 h of reperfusion (Fig. 1).

Study groups

This study consisted of five series of experiments. 'I/R': rats did not receive any remifentanil (Ultiva[™], GlaxoSmithKline, Italy) treatment during the entire experiment ($n=7$); 'Remifentanil-preconditioning (Pre-R)': rats were subjected to infusion of remifentanil ($6 \mu g \text{ kg}^{-1} \text{ min}^{-1}$) during 20 min before 30 min of occlusion followed by 10 min washout ($n=6$); 'Remifentanil-postconditioning (Post-R)': remifentanil was administered during the first 20 min of reperfusion ($n=7$); 'Long-R': remifentanil was administered from 30 min before ischemia to the first 20 min of reperfusion ($n=9$). Control sham-operated rats (Sham) were treated similarly, except that the coronary suture was not tied, and remifentanil was not administered ($n=5$).

Infarct size measurement

At the end of the reperfusion period (2 h), the heart was excised and immersed in 2 % triphenyl-tetrazolium chloride (TTC) (Sigma, USA) stain for 20 min at 37 °C. The infarct myocardium, which does not take up TTC stain when the dehydrogenase enzymes are drained off, remains pale in color. The heart was sliced and photographed, and infarct size was determined by dividing the total necrotic area of the left ventricle (LV) by the total LV area (Kang *et al.* 2006, Hwang *et al.* 2004, Gneccchi *et al.* 2006). The boundary of unstained area was traced in a blinded fashion and quantified with NIH image, version 1.61.

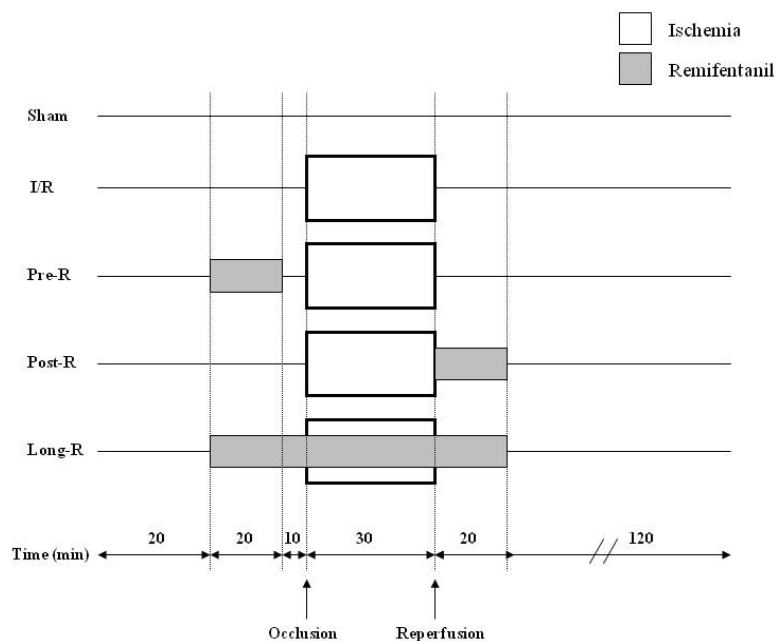


Fig. 1. The experimental protocol. Occlusion of the left anterior (LAD) coronary artery was maintained for 30 min, followed by reperfusion. After 2 h, sampling was performed for infarct size, immunoblot and RT-PCR, respectively. Sham, the coronary suture was not tied, and remifentanyl was not administered; I/R, ischemia/reperfusion group ($n=7$); Pre-R, remifentanyl administered during the preconditioning period ($n=6$); Post-R, remifentanyl administered during the first 20 min of reperfusion ($n=7$); Long-R, remifentanyl administered during the entire period (from the preconditioning to the first 20 min of reperfusion) ($n=9$).

Immunoblot analysis

At the end of the reperfusion period, tissue preparation was performed (Uecker *et al.* 2003). Briefly, LV specimens were pulverized and dissolved in lysis buffer (Cell signaling, USA). The solution was vigorously homogenized with Pyrex Potter-Elvehjem Tissue Grinders (BLD science, USA) and then centrifuged at $12,000 \times g$ for 10 min at 4°C , and supernatant was transferred to a new tube and stored at -70°C . Protein concentrations were determined using the Bradford protein assay kit (BioRad, USA). Proteins were separated in a 12% SDS-polyacrylamide gel and transferred to PVDF membrane (Millipore Co, 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 h at room temperature, membranes were washed twice with TBS-T and incubated with primary antibodies for 1 h at room temperature or for overnight at 4°C . The following primary antibodies were used: rabbit anti-extracellular signal-regulated kinases (ERK 1/2), mouse anti-phospho ERK 1/2, mouse anti-B cell leukemia/lymphoma-2 (Bcl-2), mouse anti-cytochrome C (Santa Cruz Biotechnology, USA), rabbit anti-Bcl-2-associated X protein (Bax) (Assay Designs, USA) and mouse anti- β actin antibodies (Sigma, USA). The membranes were washed three times with TBS-T for

10 min, and then incubated for 1 h 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, USA). β -actin gene was used as the standards for equal of the protein samples. The band intensities were quantified using a Photo-Image System (Molecular Dynamics, Sweden). Each experiment was performed at least three times.

RT-PCR analysis

At the end of the reperfusion period, tissue preparation was performed (Uecker *et al.* 2003). Briefly, LV samples were vigorously homogenized with Pyrex Potter-Elvehjem Tissue Grinders (BLD science, USA) in TRI Reagent (Sigma, USA). Total RNA was prepared by the UltraspectTM-II RNA system (Biotech Laboratories, Inc., USA) and single-stranded cDNA was then synthesized from isolated total RNA by Avian Myeloblastosis virus (AMV) reverse transcriptase. A $20 \mu\text{l}$ reverse transcription reaction mixture containing $1 \mu\text{g}$ 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, $0.5 \mu\text{g}$ of oligo (dT)₁₅ and 15 units of AMV reverse transcriptase were incubated at

Table 1. The nucleotide sequence of all primer pairs for proteins related to Ca²⁺ homeostasis.

Genes	Primer sequence	Size (bp)
<i>L-type Ca²⁺-channel</i>	Sense: 5'-TGTCACGGTTGGGTAGTGAA-3' Antisense: 5'-TTGAGGTGGAAGGGACTTTG-3'	346
<i>PMCA1</i>	Sense: 5'-TGCCTTGTGGGATTCTCT-3' Antisense: 5'-CACTCTGGTTCTGGCTCTCC-3'	351
<i>NCX</i>	Sense: 5'-TGTCTGCGATTGCTTGTCTC-3' Antisense: 5'-TCACTCATCTCCACCAGACG-3'	364
<i>SERCA2a</i>	Sense: 5'-TCCATCTGCCTGTCCAT-3' Antisense: 5'-GCGGTTACTCCAGTATTG-3'	196
<i>Phospholamban</i>	Sense: 5'-GCTGAGCTCCCAGACTTCAC-3' Antisense: 5'-GCGACAGCTTGTACAGAAAG-3'	339
<i>Ryanodine receptor 2</i>	Sense: 5'-CCAACATGCCAGACCCTACT-3' Antisense: 5'-TTTCTCCATCCTCTCCCTCA-3'	351
<i>Calsequestrin</i>	Sense: 5'-TCAAAGACCCACCCTACGTC-3' Antisense: 5'-CCAGTCTTCCAGCTCCTCAG-3'	352

Table 2. Hemodynamic parameters.

	Baseline		Ischemia		2 h after reperfusion	
	HR	MAP	HR	MAP	HR	MAP
<i>I/R</i>	437 ± 52	131 ± 14	446 ± 67	127 ± 17	449 ± 35	129 ± 15
<i>Pre-R</i>	456 ± 47	144 ± 7	467 ± 57	129 ± 8	462 ± 48	134 ± 10
<i>Post-R</i>	397 ± 61	129 ± 10	402 ± 48	132 ± 9	347 ± 48*	118 ± 15
<i>Long-R</i>	424 ± 38	135 ± 11	384 ± 41	101 ± 6*†	345 ± 40**†	99 ± 25†

HR, heart rate; MAP, mean arterial pressure; I/R, ischemia/reperfusion group ($n=7$); Pre-R, remifentanil administered during the preconditioning period ($n=6$); Post-R, remifentanil administered during the first 20 min of reperfusion ($n=7$); Long-R, remifentanil administered during the entire period (from the preconditioning to the first 20 min of reperfusion) ($n=9$). Data are mean ± SD. * $P<0.05$, ** $P<0.01$ vs. I/R group; † $P<0.05$, ‡ $P<0.01$ vs. baseline.

42 °C for 15 min, heated to 99 °C for 5 min, and then incubated at 0-5 °C for 5 min. All primer pairs for DNA sequencing of genes related to Ca²⁺ homeostasis are shown in Table 1. The PCR condition was 94 °C for 3 min, 94 °C for 1 min, 41-49 °C for 1 min and 72 °C for 2 min, 35 cycles, with a final extension for 10 min at 72 °C. GAPDH gene (primers 5'-accacagtcctatgccatcac-3' and 5'-tcaccaccctgtgtgctga-3', 450 bp) was used as the internal standard. The signal intensity of the amplification product was analyzed using the UVband software (UVltec, UK).

Statistical analysis

Data are presented as means ± SD or means ± SEM. Where results of blots and RT-PCR are shown, a representative experiment is depicted. Data analysis was

performed with statistical software program Prism v3.0 (GraphPad Software, USA). Hemodynamics was analyzed using two-way analysis of variance (ANOVA) with Bonferroni *post-hoc* test for multiple comparisons if F ratio was significant. Infarct sizes were analyzed between groups using ANOVA with Student-Newman-Keula *post-hoc* test for multiple comparisons. Statistical significance was defined as $P<0.05$ and $P<0.01$.

Results

Hemodynamics

The heart rate and mean arterial pressure are summarized in Table 2. The heart rates in the Post-R ($P<0.05$) and Long-R groups ($P<0.01$) were significantly lower compared to the I/R group after reperfusion. The

heart rate in the Long-R groups after reperfusion was also significantly lower compared to the baseline value ($P < 0.05$). The mean arterial pressures significantly decreased at the end of ischemia ($P < 0.01$) and after reperfusion ($P < 0.05$) compared to the baseline value in the Long-R groups. The other values of heart rate and mean arterial pressure revealed no significant differences in either inter- or intra-group comparisons.

Infarct size measurement

Mean infarct size of the LV in the I/R group was 43.6 (5.5) %. In the groups administered with remifentanyl, infarct sizes of the LV were significantly reduced to 20.1 (1.9) %, 18.8 (3.8) % and 19.3 (3.2) % in the Pre-R, Post-R and Long-R group, respectively (all $P < 0.05$). No significant differences were observed between the remifentanyl-administered groups (Fig. 2).

Western blotting analysis on phosphorylation of ERK 1/2 and apoptosis related proteins

Phosphorylations of ERK 1/2 were significantly lower in the I/R group than in the Sham group. Phosphorylations of ERK 1/2 in the remifentanyl-administered groups were significantly improved compared to the I/R group, but were lower than in the Sham group. Phosphorylations of ERK 1/2 were similar between the remifentanyl-administered groups (Fig. 3A).

Expression levels of pro-apoptotic proteins, Bax and cytochrome c, significantly increased and expression level of anti-apoptotic protein, Bcl-2 significantly decreased in the I/R group than in the Sham group. In the remifentanyl-administered groups, the expression levels of Bax and cytochrome c significantly decreased and the expression level of Bcl-2 significantly increased than in the I/R group. The expression levels of Bax, cytochrome c and Bcl-2 were similar among the remifentanyl-administered groups (Fig. 3B).

Gene expression of proteins influencing the calcium homeostasis

In the I/R group, gene expression for sarcoplasmic reticulum (SR) proteins including SR Ca^{2+} -ATPase (SERCA2a), phospholamban (PLB), ryanodine receptor 2 (RyR), and calsequestrin (CSQ) was reduced compared to Sham group. In remifentanyl-administered groups, mRNA transcription levels for SR proteins were significantly up-regulated than in the I/R group without differences between the groups. The Ca^{2+} entry across the membrane through L-type Ca^{2+} channels is balanced by the

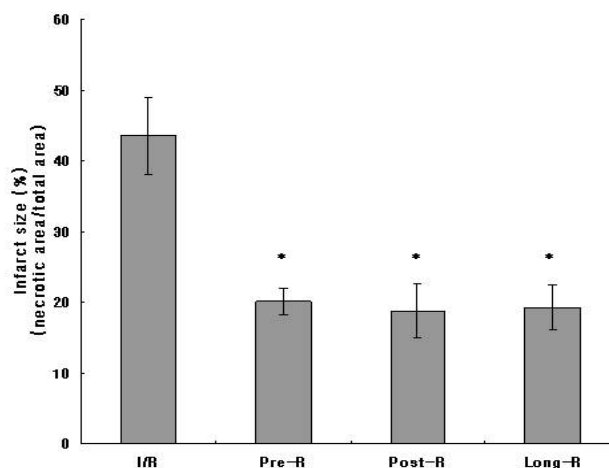


Fig. 2. Infarct sizes of the left ventricle in each group. Infarct size was expressed as a ratio of the left ventricular area. Values are presented as mean \pm SEM. * $P < 0.05$ compared with the I/R group ($n = 5-9$ per group).

efflux of Ca^{2+} from the cell via the Na^{+} - Ca^{2+} exchanger (NCX) (Bers 2002). In the present results, levels of gene expression for L-type Ca^{2+} channels and NCX were unaltered by I/R with or without remifentanyl (Fig. 4).

Discussion

In the current study, remifentanyl significantly reduced myocardial infarct size irrespective of the timing and duration of administration in I/R rat heart, which occurred in conjunction with the preserved phosphorylations of ERK 1/2 and anti-apoptotic protein Bcl2 and attenuated increased pro-apoptotic proteins, Bax and cytochrome c resulting from I/R. Remifentanyl administration also resulted in preserved gene expressions of SR proteins.

Remifentanyl is a fentanyl derivative with a unique pharmacokinetic profile of rapid and predictable recovery, gaining increasing popularity in cardiac anesthesia. Similar to certain anesthetic agent, remifentanyl has also been found to possess cardioprotective effects against I/R, although the effect occurred at significantly higher dose than that clinically used (Minto *et al.* 1997, Scott and Perry 2005). Remifentanyl was reported to exert cardioprotective effect via all three opioid receptors (Peart and Gross 2004, Yu *et al.* 2007). Activation of κ - and δ - opioid receptors was associated with activation of PKC and opening of mitochondrial K_{ATP} channel, which are important targets of cardioprotection in ischemic and pharmacological preconditioning (Zhang *et al.* 2005).

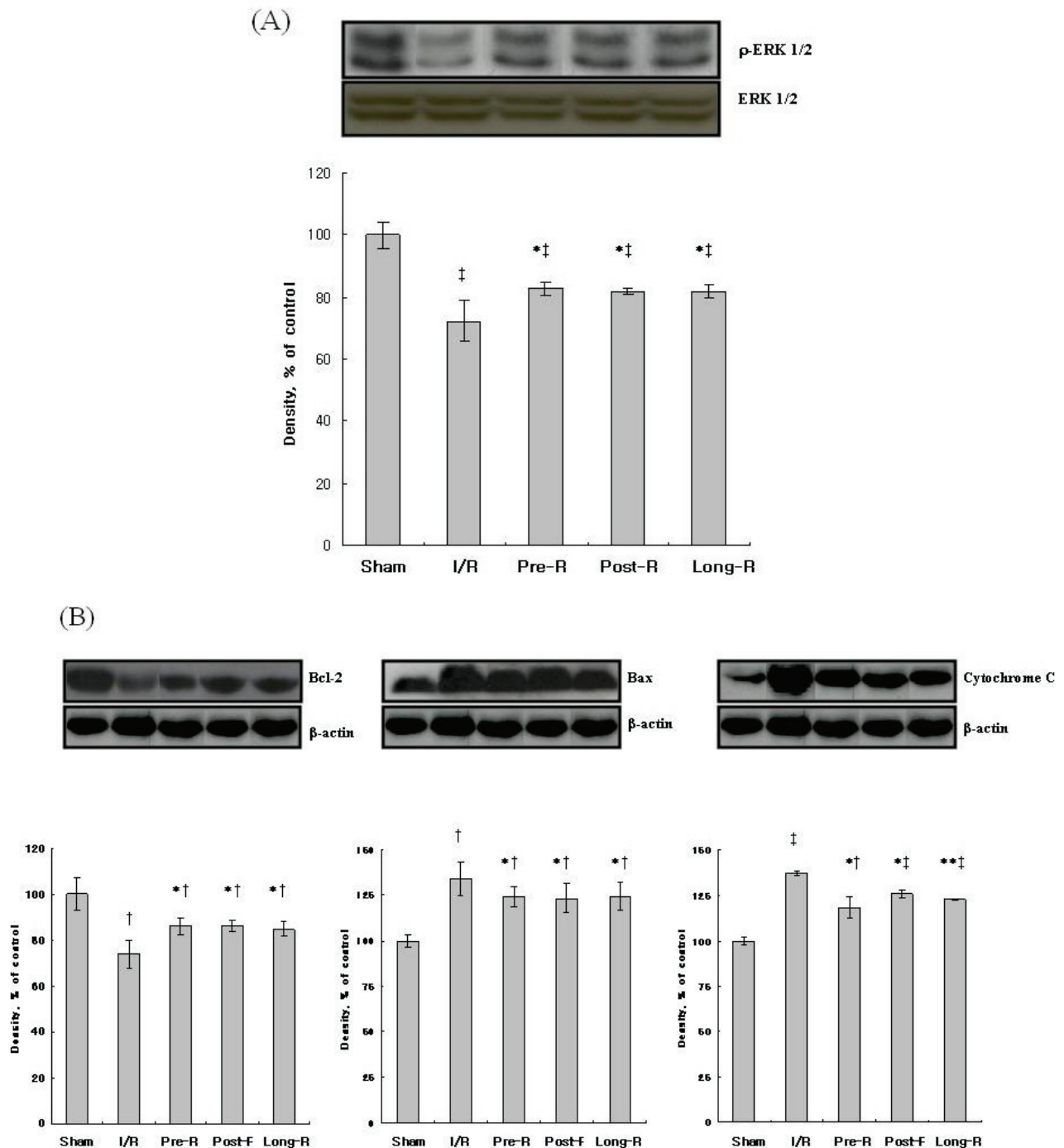


Fig. 3. Western blot analysis of (A) Western blot analysis of ERK 1/2 and p-ERK 1/2. (B) Western blot analysis of Bcl-2, Bax, and cytochrome c. Each signal was quantified by scanning densitometry. Values are presented as mean \pm SEM. * $P < 0.05$ and ** $P < 0.01$ compared with the I/R group.; † $P < 0.05$ and ‡ $P < 0.01$ compared with the Sham group.

While other signal transduction pathways also play an important role in cardioprotection, however, little has been studied regarding mechanisms of remifentanyl induced cardioprotection and we could observe that remifentanyl acted through proteins involved in anti-apoptotic pathways of survival regardless of timing and

duration of administration. Apoptosis has been linked with reperfusion-induced myocardial injury after reversible coronary occlusion and suggested as one of the key mechanisms in the development of infarction in rat cardiac myocytes (Buja and Entman 1998, Haunstetter and Izumo 2000).

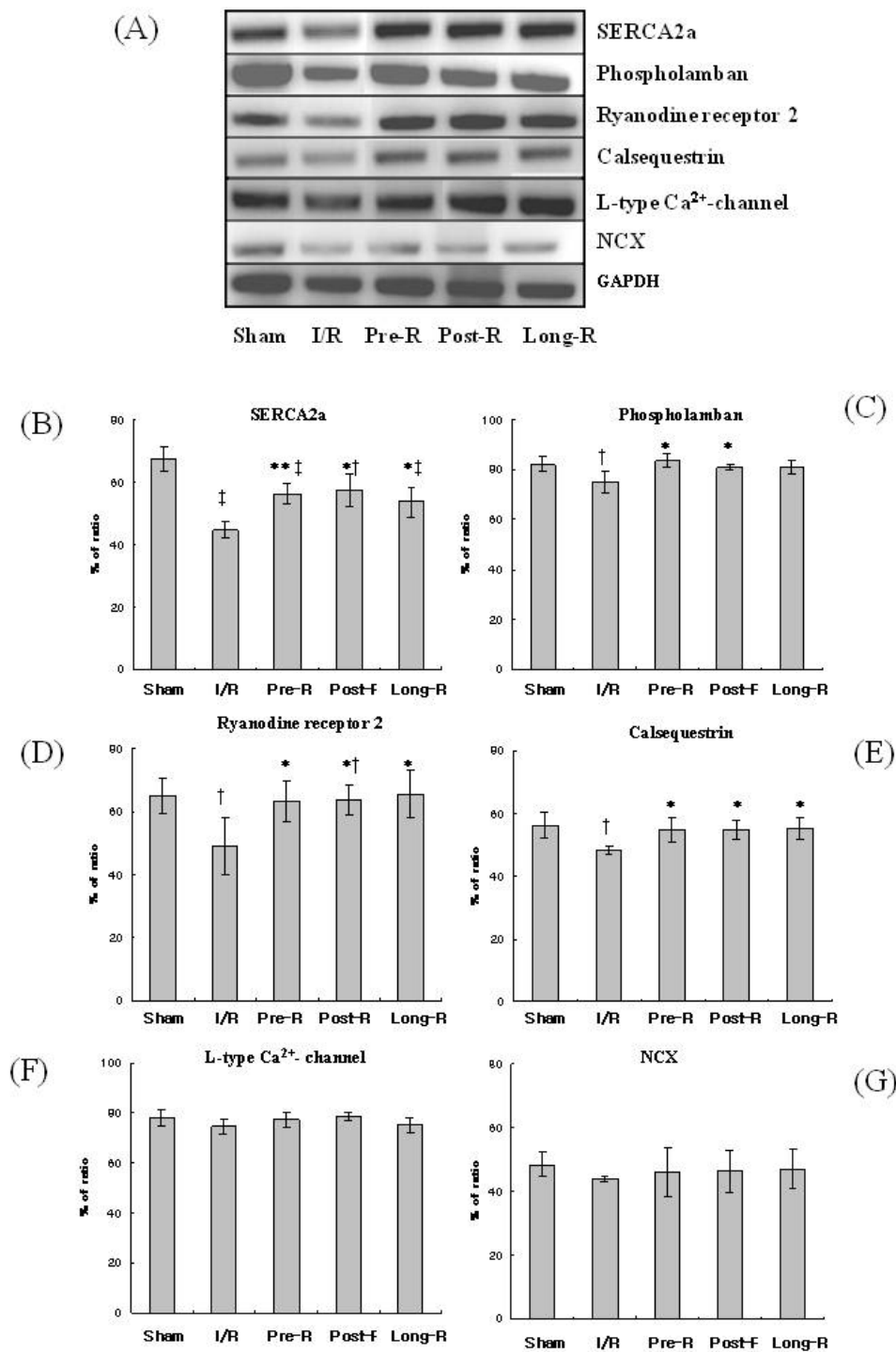


Fig. 4. Analysis of the expressions of genes related to Ca²⁺ homeostasis. **(A)** Representative gel image photographs of mRNA levels. **(B-G)** Histograms of SERCA2a **(B)**, phospholamban **(C)**, ryanodine receptor 2 **(D)**, calsequestrin **(E)**, L-type Ca²⁺-channel **(F)**, and NCX **(G)**. GAPDH was used as an internal standard. Each value is the mean \pm SEM of 5 hearts/group. * $P < 0.05$ and ** $P < 0.01$ compared with the I/R group; † $P < 0.05$ and ‡ $P < 0.01$ compared with the Sham group.

In the current study, remifentanyl increased the phosphorylation of ERK 1/2 and anti-apoptotic proteins in I/R rat heart. ERK is activated in response to I/R, oxidative stress, and hypoxia, and is an established player in the anti-apoptotic defense network (Yue *et al.* 2000).

As our results indicate, remifentanyl preserved the phosphorylation of ERK 1/2 which were significantly reduced following I/R leading to improved viability of the rat heart exposed to I/R. In response to an apoptotic stimulus such as I/R, the pro-apoptotic protein, Bax,

undergoes a conformational change that allows its translocation to the mitochondria, where it induces cytochrome c release. Phosphorylation of ERK 1/2 inhibits the conformational change in Bax protein and cytochrome c-induced caspase activation, thereby preventing apoptosis (Tsuruta *et al.* 2004). The anti-apoptotic protein Bcl-2 attenuates cellular injury by inhibiting cytochrome c translocation (Kluck *et al.* 1997) and inhibits Bax translocation (Zhu *et al.* 2001). During ischemic preconditioning, an increase in Bcl-2 in association with a decrease in the pro-apoptotic protein Bax occurred in isolated rat heart (Lazou *et al.* 2006). In this study, we could observe that improved phosphorylations of ERK1/2, and associated recovery of expression of Bcl-2 and mitigated expression of Bax and mitochondrial cytochrome c release against I/R induced injury, which all indicate remifentanyl induced a cardioprotective effect *via* anti-apoptosis pathways of survival in I/R rat heart.

Ca²⁺ is an important messenger in intracellular signal transduction and the balance between Bcl-2 and Bax has been demonstrated to affect mitochondrial Ca²⁺ homeostasis, which is important in determining whether cells survive or undergo apoptosis. The SR plays a central role in regulating the intracellular Ca²⁺ concentration and contains SR Ca²⁺-cycling proteins such as RyR, SERCA2a, PLB, and CSQ. The expression of SR genes was significantly reduced in I/R rat heart in this study. This was in accordance with the result of previous study (Temsah *et al.* 1999), and treatment with remifentanyl preserved the levels of SR genes expression. These results suggest that remifentanyl might modulate the expression of SR genes in I/R rat heart. Since we did not measure intracellular Ca²⁺ concentration, the real changes in intracellular Ca²⁺ concentration as consequences of remifentanyl-induced modulation of Ca²⁺ homeostasis could not be clarified in this study.

With regard to the effect of timing and duration of drug administration on the extent of myocardial protection, the combination of pre- and postconditioning with sevoflurane provided additive cardioprotection (Obal *et al.* 2005), whereas desflurane showed no additive cardioprotective effects (Haelewyn *et al.* 2004). As we had investigated the cardioprotective effects of remifentanyl with different administration protocols, we

observed similar protective effect between remifentanyl preconditioning and postconditioning groups, and could not observe additive cardioprotective effects in continuously administered group. These results suggest that cardioprotective signaling pathways may vary among anesthetics but activated pathways during remifentanyl pre- and postconditioning might be similar, which merit further studies addressing the precise mechanisms of this particular anesthetic-induced cardioprotection.

Cardioprotective effects of remifentanyl in this study were demonstrated at a dose of 6 µg kg⁻¹ min⁻¹ which is higher than the dose used in clinical practice (0.2-0.8 µg kg⁻¹ min⁻¹) (Scott and Perry 2005). Previous studies reported that remifentanyl had cardioprotective effect against I/R injury with dose-dependency, and the maximum effect of remifentanyl was 6 or 10 µg kg⁻¹ min⁻¹ (Yu *et al.* 2007, Zhang *et al.* 2005). Based on those studies, we choose the minimal concentration of remifentanyl inducing maximal cardioprotective effect. The pharmacological characteristics of remifentanyl may vary and it is difficult to compare between species and can be given in relatively high doses for a short period of time (Yu *et al.* 2007, Zhang *et al.* 2005).

The limitation of this study is that we did not determine the area at risk. In this study, we referred the infarct size measurement to the methods of our research (Hwang *et al.* 2004, Kang *et al.* 2006). Therefore we determined the infarct size by dividing the total necrotic area by the total LV area. Although this method was used in other study (Bazargan *et al.* 2008), determining the necrotic area by the area at risk can be more accurate.

In conclusion, remifentanyl confers myocardial protection against injury in I/R rat heart without reference to the timing and duration of administration, which was conducted with preserved phosphorylations of ERK 1/2 and of signal transduction proteins against apoptotic cell death, and attenuated changes in expression of SR genes related to Ca²⁺ homeostasis.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the Brain Korea 21 Project for Medical Science, Yonsei University, Korea.

References

- ANAYA-PRADO R, TOLENDO-PEREYRA LH: The molecular events underlying ischemia/reperfusion injury. *Transplant Proc* **34**: 2518-2519, 2002.

- BAZARGAN M, FAGHIHI M, CHITSAZ M: Importance of timing of magnesium administration in the isolated ischemic-reperfused rat heart: role of K_{ATP} channels. *Physiol Res* **57**: 839-846, 2008.
- BERS DM: Calcium and cardiac rhythms: physiological and pathophysiological. *Circ Res* **90**: 14-17, 2002.
- FLISS H, GATTINGER D: Apoptosis in ischemic and reperfused rat myocardium. *Circ Res* **79**: 949-956, 1996.
- GLASS PS: Remifentanyl: a new opioid. *J Clin Anesth* **7**: 558-563, 1995.
- GOTTLIEB RA, ENGLER RL: Apoptosis in myocardial ischemia-reperfusion. *Ann N Y Acad Sci* **874**: 412-426, 1999.
- HAELEWYN B, ZHU L, HANOUS JL, PERSEHAYE E, ROUSSEL S, DUCOURET P, GERARD JL: Cardioprotective effects of desflurane: effect of timing and duration of administration in rat myocardium. *Br J Anaesth* **92**: 552-557, 2004.
- HWANG KC, LIM S, KWON HM, BAE YS, KANG SM, CHUNG KH, GRAHAM RM, RHEE SG, JANG Y: Phospholipase C-delta1 rescues intracellular Ca^{2+} overload in ischemic heart and hypoxic neonatal cardiomyocytes. *J Steroid Biochem Mol Biol* **91**: 131-138, 2004.
- KANG SM, LIM S, SONG H, CHANG W, LEE S, BAE SM, CHUNG JH, LEE H, KIM HG, YOON DH, KIM TW, JANG Y, SUNG JM, CHUNG NS, HWANG KC: Allopurinol modulates reactive oxygen species generation and Ca^{2+} overload in ischemia-reperfused heart and hypoxia- reoxygenated cardiomyocytes. *Eur J Pharmacol* **535**: 212-219.
- KLUCK RM, BOSSY-WETZEL E, GREEN DR, NEWMAYER DD: The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* **275**: 1132-1136, 1997.
- LAZOU A, ILIODROMITIS EK, CIESLAK D, VOSKARIDES K, MOUSIKOS S, BOFILIS E, KREMASTINOS DT: Ischemic but not mechanical preconditioning attenuates ischemia/reperfusion induced myocardial apoptosis in anaesthetized rabbits: the role of Bcl-2 family proteins and ERK1/2. *Apoptosis* **11**: 2195-2204, 2006.
- MINTO CF, SCHNIDER TW, SHAFER SL: Pharmacokinetics and pharmacodynamics of remifentanyl. *Anesthesiology* **86**: 24-33, 1997.
- MURRY CE, JENNINGS RB, REIMER KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* **74**: 1124-1136, 1986.
- OBAL D, DETTWILER S, FAVOCCIA C, SCHARBATKE H, PRECKEL B, SCHLACK W: The influence of mitochondrial KATP-channels in the cardioprotection of preconditioning and postconditioning by sevoflurane in the rat in vivo. *Anesth Analg* **101**: 1252-1260, 2005.
- PATEL SS, SPENCER CM: Remifentanyl. *Drugs* **52**: 417-427, 1996.
- PEART JN, GROSS ER, GROSS GJ: Opioid-induced preconditioning: recent advances and future perspectives. *Vascul Pharmacol* **42**: 211-218, 2005.
- PEART JN, GROSS GJ: Exogenous activation of delta- and kappa-opioid receptors affords cardioprotection in isolated murine heart. *Basic Res Cardiol* **99**: 29-37, 2004.
- SCHULTZ JE, GROSS GJ: Opioids and cardioprotection. *Pharmacol Ther* **89**: 123-137, 2001.
- SCOTT LJ, PERRY CM: Spotlight on remifentanyl for general anaesthesia. *CNS Drugs* **19**: 1069-1074, 2005.
- TANAKA K, LUDWIG LM, KERSTEN JR, PAGEL PS, WARLTIER DC: Mechanisms of cardioprotection by volatile anesthetics. *Anesthesiology* **100**: 707-721, 2004.
- TEMSAH RM, NETTICADAN T, CHAPMAN D, TAKEDA S, MOCHIZUKI S, DHALLA NS: Alterations in sarcoplasmic reticulum function and gene expression in ischemic-reperfused rat heart. *Am J Physiol* **277**: H584-H594, 1999.
- TSURUTA F, MASUYAMA N, GOTOH Y: The phosphatidylinositol 3-kinase (PI3K)-Akt pathway suppresses Bax translocation to mitochondria. *J Biol Chem* **277**: 14040-14047, 2002.
- UECKER M, DA SILVA R, GRAMPP T, PASCH T, SCHAUB MC, ZAUGG M: Translocation of protein kinase C isoforms to subcellular targets in ischemic and anesthetic preconditioning. *Anesthesiology* **99**: 138-147, 2003.
- YU CK, LI YH, WONG GT, WONG TM, IRWIN MG: Remifentanyl preconditioning confers delayed cardioprotection in the rat. *Br J Anaesth* **99**: 632-638, 2007.
- YUE TL, WANG C, GU JL, MA XL, KUMAR S, LEE JC, FEUERSTEIN GZ, THOMAS H, MALEEFF B, OHLSTEIN EH: Inhibition of extracellular signal-regulated kinase enhances ischemia/reoxygenation-induced apoptosis in cultured cardiac myocytes and exaggerates reperfusion injury in isolated perfused heart. *Circ Res* **86**: 692-699, 2000.

-
- ZHANG Y, CHEN ZW, GIRWIN M, WONG TM: Remifentanyl mimics cardioprotective effect of ischemic preconditioning via protein kinase C activation in open chest of rats. *Acta Pharmacol Sin* **26**: 546-550, 2005.
- ZHANG Y, IRWIN MG, WONG TM: Remifentanyl preconditioning protects against ischemic injury in the intact rat heart. *Anesthesiology* **101**: 918-923, 2004.
- ZHU L, YU Y, CHUA BH, HO YS, KUO TH: Regulation of sodium-calcium exchange and mitochondrial energetics by Bcl-2 in the heart of transgenic mice. *J Mol Cell Cardiol* **33**: 2135-2144, 2001.
-