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Remifentanil postconditioning has cross talk with adenosine receptors in the ischemic-reperfused rat heart

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ABSTRACT

Background: Although there is a possibility of cross talk between opioid and adenosine signaling pathways in the ischemic-reperfused myocardium, it is not clear that an ultra-short–acting opioid receptor agonist remifentanil-induced postconditioning (RPostC) has cross talk with adenosine receptor (ADR). The purpose of this study was to determine whether there is cross talk with ADR in RPostC.

Materials and methods: Isolated rat hearts were subjected to 30 min of regional ischemia and 2 h of reperfusion. RPostC was induced by 100 ng/mL of remifentanil perfusion, 5 min before reperfusion, followed by 5 min of reperfusion. The nonspecific opioid receptor antagonist naloxone (NAL) and the nonspecific ADR antagonist 8-(p-sulfophenyl) theophylline hydrate (8-SPT) were perfused for a 20-min period, 10 min before RPostC to the end of RPostC. Western blot analysis was performed to detect phospho-ERK1/2 in cultured cardiomyocytes.

Results: In cultured cardiomyocytes, remifentanil incubation significantly increased the phosphorylation of ERK1/2 and this effect was blocked by both NAL and 8-SPT ($P < 0.01$ and $P < 0.05$, respectively). RPostC significantly reduced infarct size over ischemic area at risk from 34.1 ± 10.5% to 16.6 ± 7.5% ($P < 0.05$ *versus* control). The infarct-limitation effect of RPostC was reversed by both NAL (33.8 ± 13.0%, $P < 0.05$) and 8-SPT (35.7 ± 14.5%, $P < 0.01$).

Conclusions: This study strongly implies that the intracellular signaling pathways of cardioprotection by RPostC has cross talk with ADR in the ischemic-reperfused myocardium.

1. Introduction

Remifentanil, a fentanyl derivative, is commonly used in anesthesia for several branches of surgery. Remifentanil is an ultra-short–acting phenylpiperidine opioid and a nonspecific opioid receptor (OPR) agonist. Remifentanil-induced preconditioning and postconditioning have been demonstrated to reduce the infarct size against myocardial

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ischemia–reperfusion (I/R) injury via the activation of OPR, especially κ- or σ- OPR [1,2].

Meanwhile, the signaling pathways responsible for OPR- and adenosine receptor (ADR)-mediated cardioprotection seem to be similar. Interestingly, it has been proposed that there is cross talk between opioid and adenosine signaling pathways in I/R-injured myocardium. For example, a potent OPR agonist fentanyl administered before induction of ischemia and maintained throughout the reperfusion protected the heart against postischemic injury by ADR as well as OPR [3]. A selective A_1 ADR antagonist attenuated the postischemic mechanical function by the OPR agonist fentanyl in isolated-perfused rat heart. In addition, there is a report that the effects of pharmacologic preconditioning by the nonselective OPR agonist morphine and the selective A_1 ADR agonist were attenuated by the pretreatment of a selective A_1 ADR antagonist and a selective δ-OPR antagonist, respectively [4], which means morphine-targeting ischemia has cross talk with ADR.

Most of the previous report about the receptor cross talk in myocardial I/R injury was targeted ischemic period; moreover, there is scanty information whether the remifentanil-induced cardioprotection targeted reperfusion period has cross talk with ADR. Therefore, in this study, we tried to determine whether there is cross talk with ADR in remifentanil-induced postconditioning (RPostC).

2. Materials and methods

All experimental procedures and animal care were performed in accordance with the International Guiding Principles for Biomedical Research Involving Animals. The experimental procedures and protocols used in this study were reviewed and approved by Institutional Animal Care and Use Committee.

2.1. *Isolation of adult rat cardiomyocytes*

Rat cardiomyocytes were isolated enzymatically [5]. The isolated heart was perfused in a nonrecirculating mode with Krebs–Henseleit (KH) buffer for 5 min to wash out blood and was perfused with a calcium-free buffer. After 5 min of perfusion, collagenase (type 2) was added to the buffer (0.1%) and the heart was perfused in a recirculating mode for about 15 min. The heart was removed from the apparatus and the ventricles were placed into a beaker containing the calcium-free buffer. The ventricles were agitated in a shaking bath (37°C) at a rate of 50 cycles per min until individual cells were released. The released cells were suspended in an incubation buffer containing all the components of the calcium-free buffer, 1% bovine serum albumin, 30 mM HEPES, 60 mM taurine, 20 mM creatine, and amino-acid supplements at 37°C. Calcium was gradually added to the buffer-containing cells to a final concentration of 1.2 mM. The cells were filtered through a nylon mesh and centrifuged briefly. Finally, the cells were suspended in serum-free Dulbecco's Modified Eagle's Medium for 1 h before the experiments (Fig. 1).

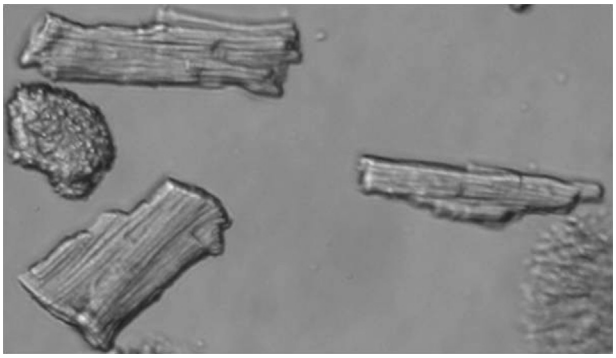

Fig. 1 – Isolated cardiomyocytes from a rat. The viability of cardiomyocytes (rod shape: viable, round shape: dead) was over 85% when checked under a microscope according to their morphology (×200).

2.2. *Western immunoblottings*

To determine the change of ERK1/2 (Thr202/Tyr204) expression by pretreatment of nonspecific OPR antagonist naloxone (NAL) or nonspecific ADR antagonist 8-(p-sulfophenyl) theophylline hydrate (8-SPT) in remifentanil-incubated cardiomyocytes, phosphorylation of ERK1/2 was measured. Cardiomyocytes were pretreated with NAL or 8-SPT for 10 min and then incubated with remifentanil for further 30 min. Phosphate-buffered saline-treated cells at the same duration were used as a control. Cardiomyocytes were collected and lysed in ice-cold RIPA buffer (25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) containing protease inhibitor and phosphatase inhibitor cocktails (Sigma–Aldrich Chemical, St. Louis, MO). Protein concentration was measured by a bicinchoninic acid method according to the manufacturer's instruction (Pierce, Appleton, WI). Equal amounts of protein (80 μg) were loaded and electrophoresed on 10% SDS–polyacrylamide gel and transferred to a polyvinylidene fluoride membrane. Membranes were blocked with nonfat milk and then incubated with the primary antibodies (1:1,000; Cell Signaling Technologies, Beverly, MA) at 4°C overnight. The primary antibody bindings were detected with a secondary anti-rabbit antibody (1:2000) and visualized by the enhanced chemiluminescence method. After detecting phospho-of ERK1/2, polyvinylidene fluoride membranes were stripped and reprobed with their total forms of antibody. In addition, equal loading of samples were confirmed by reprobing membranes with anti-beta tubulin. The band density from Western blotting was measured using Multi Gauge version 3.0 (Fujifilm, Tokyo, Japan) software. Phosphorylation rate of ERK1/2 were calculated from their total forms and expressed as a percentage of control.

2.3. *Langendorff-isolated rat heart perfusion preparation*

Male Sprague–Dawley rats (KOATECH Co, Cheongwon-gun, Republic of Korea) weighing 300–350 g received 50 mg/kg of pentobarbital sodium and 300 IU of heparin intraperitoneally.