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Remifentanil Preconditioning Confers Cardioprotection via Cardiac κ- and δ-Opioid Receptors

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Background: **Remifentanil preconditioning (RPC) reduces the infarct size in anesthetized rat hearts, and this effect seems to be mediated by all three types of opioid receptors (ORs). Because there is evidence of only κ- and δ- but not μ-ORs in the rat heart, the authors investigated whether RPC confers cardioprotection *via* cardiac κ- and δ-OR as well as *via* extracardiac μ-OR agonist activity. The authors also investigated the involvement of signaling mechanisms, namely protein kinase C and mitochondrial adenosine triphosphate–sensitive potassium (K_{ATP}) channels.**

Methods: **The hearts of male Sprague-Dawley rats weighing 190–210 g were removed, mounted on a Langendorff apparatus, and perfused retrogradely at 100 cm H_2O with Krebs-Ringer's solution. All hearts were subjected to 30 min of ischemia and 2 h of reperfusion. The study consisted of three series of experiments on the effect of ischemic preconditioning or RPC (10, 50, and 100 ng/ml remifentanil) after blockade of OR subtypes (δ-OR antagonist naltrindol, κ-OR antagonist nor-binaltorphimine, and μ-OR antagonist CTOP). The involvement of protein kinase C or the K_{ATP} channel in the cardioprotection of RPC was also investigated using specific blockers in each group. RPC was produced by three cycles of 5-min perfusion of remifentanil in Krebs-Ringer's solution interspersed with a 5-min reperfusion with Krebs solution only. Infarct size, as a percentage of the area at risk, was determined by 2,3,5-triphenyltetrazolium staining.**

Results: **Infarct size as a percentage of the area at risk was significantly reduced after RPC from 51.9 ± 5.0% (control, n = 8) to 36.2 ± 10.0% (100 ng/ml RPC, n = 8, $P < 0.01$). This effect was stopped by pretreatment with naltrindol (52.3 ± 5.2%) and nor-binaltorphimine (43.5 ± 6.0%) but not CTOP (37.1 ± 6.0%). Chelerythrine and GF109203X, both protein kinase C inhibitors, abolished the effects of RPC or ischemic preconditioning on infarct size as a percentage of area at risk. 5-Hydroxydecanoate (a selective mitochondrial K_{ATP} channel blocker) also abolished the cardioprotection of RPC and IPC, but HMR-1098 (a selective inhibitor of the sarcolemmal K_{ATP} channel) did not.**

Conclusion: **Cardiac δ- and κ- but not μ-ORs mediate the cardioprotection produced by RPC. Both protein kinase C and the mitochondrial K_{ATP} channel were involved in this effect.**

IN a previous study, we showed that administration of remifentanil for three cycles of 5 min each interspersed with 5 min of reperfusion reduced the infarct size (IS) in the heart of anesthetized rats, indicating cardioprotection, an effect similar to that of ischemic preconditioning (IPC).[1] Interestingly, the cardioprotective effect of remifentanil preconditioning (RPC) was abolished by systemic administration of κ- or δ- or μ-opioid receptor (OR) antagonists, indicating that all three types of ORs mediate the cardioprotection of RPC.[1] Previous binding[2–5] and functional[6,7] studies have shown that in the heart there are κ- and δ- but not μ-ORs. It has also been shown that morphine affects cardiac function *via* κ- and δ-ORs, although it is a strong μ-OR agonist.[8] Therefore, it is hypothesized that remifentanil, which has a high degree of μ-OR selectivity (EC_{50} = 2.6 nM) with a lower affinity for δ-OR (EC_{50} = 66 nM) and κ-OR (EC_{50} = 6.1 μM),[9] may confer cardioprotection *via* cardiac κ- and δ-ORs as well as *via* extracardiac μ-OR.

The purpose of the current study, therefore, was to test this hypothesis. An isolated perfused rat heart preparation was used because this *in vitro* preparation would enable us to test the hypothesis. Three series of experiments were performed. In the first, we determined the effect of RPC on postischemic myocardial injury in isolated rat hearts. Second, we determined the effect of RPC or IPC in the isolated heart subjected to ischemia and reperfusion on blockade of ORs with selective OR antagonists. Last, we also determined the involvement of signaling mechanisms, namely protein kinase C (PKC) and sarcolemmal (sarc-K_{ATP}) and/or mitochondrial (mito-K_{ATP}) adenosine triphosphate–sensitive potassium (K_{ATP}) channels, known to mediate the cardioprotection produced by both IPC and *via* activation of κ- and δ-ORs.[10–15]

Materials and Methods

This study was conducted in accordance with our institutional guidelines on the use of live animals for research, and the experimental protocol was approved by the Animal Care and Use Committee of the University of Hong Kong.

Isolated Heart Preparation

Our preparation and measurements have been described previously in detail.[14,16] Briefly, male Sprague-Dawley rats weighing 190–210 g were killed by decapitation with a guillotine. The heart was removed immediately and mounted to Langendorff apparatus and perfused retrogradely at 100 cm H_2O with Krebs-Ringer's solution containing 115 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 1.25 mM $CaCl_2$, 25 mM $NaHCO_3$, and

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11 mM glucose. The solution was oxygenated with a mixture of 95% oxygen and 5% carbon dioxide, which kept the pH at 7.4. The solution temperature was maintained at 37°C by a temperature-regulating device (Grant; Grant Instruments Ltd., Cambridge, United Kingdom). Heart rate (HR) was recorded *via* electrocardiogram by PowerLab Systems (ML750 PowerLab/4sp; AD Instruments, Colorado Springs, CO). Total coronary arterial flow was measured by timed collection of the coronary venous effluent in a graduated cylinder. A 3-0 silk thread was passed around the left main coronary artery along with a snare occluder placed at the origin of the left coronary artery. Regional ischemia was achieved by pulling the snare and securing the threads with a mosquito hemostat. Myocardial ischemia was confirmed by regional cyanosis and a substantial decrease in coronary flow (CF). Reperfusion was achieved by releasing the snare. In the first 15 min of perfusion, the heart was allowed to stabilize, and any heart exhibiting arrhythmia during this period was discarded.

Remifentanil Preconditioning

Remifentanil preconditioning was produced by three cycles of 5-min perfusion of remifentanil (GlaxoSmith-Kline Limited, Hong Kong) in Krebs-Ringer's solution interspersed with 5 min of reperfusion with Krebs solution only.

Ischemic Preconditioning

The heart was subjected to three cycles of 5-min occlusion periods interspersed with 5 of min reperfusion.

Study Groups

The current study consisted of three series of experiments (fig. 1). All hearts were subjected to 30 min of regional ischemia and 120 min of reperfusion after a 15-min stabilization period. To determine whether the administration of remifentanil limits myocardial IS in the isolated rat heart, hearts were randomly assigned to receive one of four treatments: control, or RPC using one of three drug concentrations: 10, 50, or 100 ng/ml. These experiments are referred to as series 1.

In the second series of experiments, in which the effects of IPC or RPC were determined on blockade of OR subtypes, hearts were randomly assigned to 1 of 10 groups:

1. naltrindol, a selective δ-OR antagonist, 5×10^{-6} mM
2. nor-binaltorphimine, a κ-OR selective antagonist, 5×10^{-6} mM
3. D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP), a μ-OR selective antagonist, 5×10^{-6} mM
4. IPC
5. naltrindol + RPC
6. naltrindol + IPC
7. nor-binaltorphimine + RPC

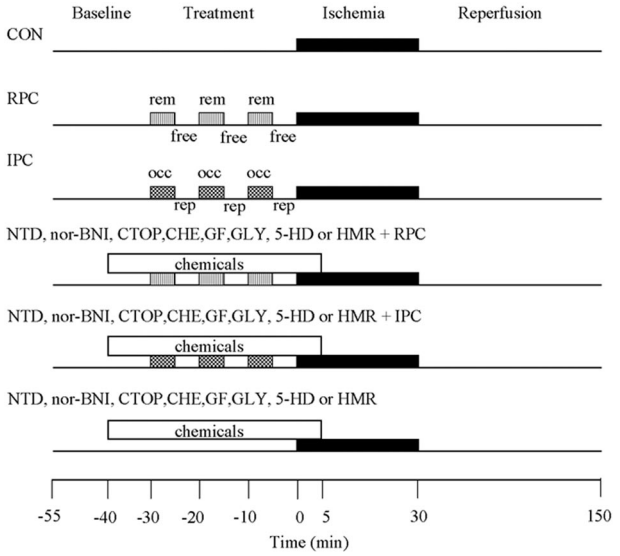

Fig. 1. Hearts in all groups were subject to 30 min of coronary artery occlusion (occ) and 120 min of reperfusion (rep). Remifentanil preconditioning (RPC) group hearts were subject to three cycles of 5-min perfusion of reminfentanil (rem) periods interspersed with drug-free periods (free). Ischemic preconditioning (IPC) hearts were subject to three cycles of 5-min coronary artery occlusion interspersed with 5-min reperfusion. Naltrindole (NTD, 5×10^{-6} M), nor-binaltorphimine (BNI, 5×10^{-6} M), CTOP (5×10^{-6} M), chelerythrine (CHE, 5×10^{-6} M), GF109203X (GF 1×10^{-5} M), glibenclamide (GLY, 1×10^{-5} M), 5-hydroxydecanoate (5-HD, 1×10^{-4} M), and HMR-1098 (HMR 1×10^{-4} M).

8. nor-binaltorphimine + IPC
9. CTOP + RPC
10. CTOP + IPC

The chemicals (all purchased from Sigma Chemical Company, St. Louis, MO) were perfused for a period of 10 min before RPC or IPC to 5 min after the end of RPC or IPC, respectively. The concentrations of all chemicals, naltrindol,[14,16] nor-binaltorphimine,[14,16] and CTOP,[17] used in this study were based on our previous studies.[1]

The third series of experiments was performed to determine the involvement of PKC or the K_{ATP} channel in the cardioprotection of RPC. Hearts were randomly assigned to 1 of 15 groups:

1. chelerythrine, a PKC inhibitor, purchased from Sigma Chemical Company
2. GF109203X, a PKC inhibitor, purchased from Tocris Cookson Ltd. (Bristol, United Kingdom)
3. glibenclamide, a nonselective blocker of the K_{ATP} channel, purchased from Sigma Chemical Company, dissolved in dimethyl sulfoxide to a final concentration of less than 0.1%
4. 5-hydroxydecanoate, a selective inhibitor of mito-K_{ATP} channels, purchased from Sigma Chemical Company
5. HMR-1098, a selective inhibitor of the sarc-K_{ATP} channel, donated by Aventis Pharma Deutschland GmbH (Frankfurt, Germany)
6. chelerythrine + RPC

7. chelerythrine + IPC
8. GF109203X + RPC
9. GF109203X + IPC
10. glibenclamide + RPC
11. glibenclamide + IPC
12. 5-hydroxydecanoate + RPC
13. 5-hydroxydecanoate + IPC
14. HMR-1098 + RPC
15. HMR-1098 + IPC

Chemicals were perfused for a period of 10 min before RPC or IPC until 5 min after the end of RPC or IPC, respectively. These experiments are referred to as series 3. The concentrations of all chemicals, chelerythrine (5×10^{-6} mM),[14,16] GF109203X (1×10^{-5} mM),[16] glibenclamide (1×10^{-5} mM),[16] 5-hydroxydecanoate (1×10^{-4} mM),[14,16,18] and HMR-1098 (1×10^{-4} mM),[18] used in this study were based on our previous studies.

Determination of Infarct Size

After the experiment, the snare was securely retightened, and 0.25% Evan blue dye was injected to stain the normally perfused region of the heart. This procedure allowed visualization of the normal, nonischemic region and the area at risk (AAR). The heart was then weighed, frozen, and cut into 2-mm slices. Thereafter, the slices were stained by incubation at 37°C for 20 min in 1% 2,3,5-triphenyltetrazolium (Sigma Chemical Co.) in phosphate buffer (pH 7.4) and then immersed in 10% formalin to enhance the contrast of the stain. The areas of infarct (2,3,5-triphenyltetrazolium negative) and risk zone (2,3,5-triphenyltetrazolium stained) for each slice were traced and digitized using a computerized planimetry technique (SigmaScan 4.0; Systat Software, San Diego, CA). The volumes of the IS and AAR were calculated by multiplying each area with slice thickness and summing the product. The IS was expressed as a percentage of the AAR (IS/AAR).

Determination of Myocardial Injury via *LDH Efflux*

The measurement has been described previously.[16] The coronary effluent was collected at 5 and 10 min after reperfusion. LDH was spectrophotometrically assayed using a kit purchased from Stanbio Laboratory (Boerne, TX). LDH activity was expressed as units per liter.

Statistical Analysis

Data analysis was performed with a personal computer statistical software package (Prism version 4.0; GraphPad Software, San Diego, CA). Data were expressed as mean ± SD. Hemodynamics were analyzed using two-way analysis of variance with Bonferroni *post hoc* test for multiple comparisons if significant F ratios were obtained. IS values (expressed as percentage of the AAR) were analyzed between groups using analysis of variance with a Student–Newman–Keuls *post hoc* test for multiple comparisons. Statistical differences were considered significant if the P value was less than 0.05.

Results

A total of 206 hearts were used in the study. Six hearts were omitted from further data analysis because coronary flow was more than 15 ml/min. Subsequently, one heart in each of the following seven groups was excluded because of intractable ventricular fibrillation: naltrindol + RPC, GF109203X + IPC, 5-hydroxydecanoate + RPC, naltrindol + IPC, chelerythrine + IPC, 5-hydroxydecanoate + IPC, and chelerythrine. One heart in each of the following four groups was excluded because of an excessively large AAR volume (greater than 0.550 mm^3): glibenclamide + RPC, 5-hydroxydecanoate + RPC, nor-binaltorphimine, and 5-hydroxydecanoate. A total of 189 hearts were studied completely.

Effects of RPC on Myocardial Infarct Size, LDH, and Hemodynamics after Ischemia and Reperfusion

The morphometrics of the groups are shown in table 1. Among all groups, there were no significant differences in left and right ventricular volumes and AAR (all $P > 0.05$). As shown in figure 2 the IS, expressed as a percentage of the AAR, of the control group was 51.9 ± 5.0% (n = 8). The maximum reduction, 36.2 ± 10.0%, was produced at 100 ng/ml. Therefore, in subsequent studies on blockade of the effects of RPC, a concentration of 100 ng/ml was used.

Remifentanil preconditioning also reduced the release of LDH during reperfusion (fig. 3). At 5 min into reperfusion, the effects of all three concentrations (10, 50, and 100 ng/ml) were significant, whereas at 10 min into reperfusion, only the effects of 50 and 100 ng/ml were significant.

Although there were slight but significant reductions in HR and slight but significant increases in CF during the preconditioning period, there were no significant differences between groups during ischemia and reperfusion in HR and CF (table 2).

Effects of RPC or IPC on Infarct Size and LDH in the Isolated Perfused Rat Heart Subject to Ischemia and Reperfusion with Blockade of an Opioid Receptor

Ischemic preconditioning and RPC (100 ng/ml markedly reduced IS/AAR from 51.9 ± 5.0% (n = 8) to 12.9 ± 3.4% (n = 8; $P < 0.01$ *vs.* control) and 36.2 ± 10.0% (n = 8; $P < 0.01$ *vs.* control), respectively. Naltrindol (5×10^{-6} mM), a selective δ-OR blocker, or nor-binaltorphimine (5×10^{-6} mM), a selective κ-OR blocker, but not CTOP (5×10^{-6} mM), a selective μ-OR blocker, completely abolished the infarct-sparing effects of RPC and IPC (figs. 4A and B).

Table 1. Morphometrics: Remifentanil Preconditioning and Ischemia Preconditioning

Treatment	n	Body Weight, g	Heart Weight, g	LV + RV Volume, cm^3	AAR Volume, cm^3
CON	8	198 ± 3	0.86 ± 0.11	0.78 ± 0.08	0.410 ± 0.076
RPC10	8	201 ± 5	0.88 ± 0.12	0.85 ± 0.10	0.423 ± 0.048
RPC50	8	204 ± 4	1.08 ± 0.09	0.81 ± 0.11	0.388 ± 0.052
RPC100	8	199 ± 3	0.93 ± 0.09	0.83 ± 0.10	0.394 ± 0.046
NTD + RPC	7	195 ± 8	0.82 ± 0.09	0.77 ± 0.09	0.371 ± 0.053
BNI + RPC	8	196 ± 7	0.83 ± 0.08	0.71 ± 0.09	0.369 ± 0.023
CTOP + RPC	8	197 ± 6	0.85 ± 0.10	0.77 ± 0.05	0.415 ± 0.038
IPC	8	203 ± 5	0.95 ± 0.07	0.81 ± 0.12	0.388 ± 0.036
NTD + IPC	7	192 ± 4	0.93 ± 0.07	0.79 ± 0.08	0.416 ± 0.032
BNI + IPC	8	193 ± 8	0.95 ± 0.09	0.87 ± 0.04	0.386 ± 0.051
CTOP + IPC	8	195 ± 6	0.85 ± 0.08	0.82 ± 0.09	0.451 ± 0.031
NTD	6	202 ± 5	0.95 ± 0.07	0.75 ± 0.10	0.375 ± 0.064
BNI	5	199 ± 6	0.91 ± 0.10	0.75 ± 0.11	0.420 ± 0.031
CTOP	5	201 ± 6	0.89 ± 0.09	0.78 ± 0.11	0.380 ± 0.059
CHE + RPC	8	209 ± 3	0.88 ± 0.08	0.81 ± 0.08	0.381 ± 0.048
GF + RPC	7	197 ± 6	0.89 ± 0.07	0.79 ± 0.06	0.403 ± 0.045
GLY + RPC	7	195 ± 7	0.90 ± 0.08	0.84 ± 0.07	0.402 ± 0.037
5-HD + RPC	6	201 ± 4	1.03 ± 0.06	0.87 ± 0.03	0.379 ± 0.042
HMR + RPC	8	199 ± 5	0.97 ± 0.08	0.78 ± 0.05	0.387 ± 0.049
CHE + IPC	7	206 ± 5	0.98 ± 0.07	0.82 ± 0.05	0.384 ± 0.044
GF + IPC	8	195 ± 5	0.91 ± 0.08	0.81 ± 0.09	0.404 ± 0.040
GLY + IPC	8	197 ± 4	0.90 ± 0.10	0.74 ± 0.05	0.382 ± 0.038
5-HD + IPC	7	194 ± 7	0.97 ± 0.07	0.80 ± 0.07	0.413 ± 0.025
HMR + IPC	8	201 ± 4	0.88 ± 0.09	0.84 ± 0.05	0.402 ± 0.037
CHE	5	202 ± 3	0.87 ± 0.07	0.80 ± 0.05	0.370 ± 0.042
GF	6	196 ± 4	0.92 ± 0.09	0.84 ± 0.06	0.383 ± 0.049
GLY	6	195 ± 3	1.01 ± 0.08	0.78 ± 0.07	0.401 ± 0.034
5-HD	5	197 ± 4	0.95 ± 0.09	0.79 ± 0.06	0.406 ± 0.020
HMR	6	199 ± 3	0.93 ± 0.08	0.82 ± 0.04	0.396 ± 0.031

Values are presented as mean ± SD. Morphometrics in rat hearts subjected to control (CON), naltrindol (NTD, 5×10^{-6} M), nor-binaltorphimine (BNI, 5×10^{-6} M), CTOP (1×10^{-6} M), chemerythrine (CHE, 5×10^{-6} M), GF109203X (GF, 1×10^{-5} M), glibenclamide (GLY, 1×10^{-5} M), 5-hydroxydecanoate (5-HD, 1×10^{-4} M), HMR-1098 (HMR, 1×10^{-4} M), remifentanil preconditioning (RPC), ischemic preconditioning (IPC), NTD + RPC or IPC, BNI + RPC or IPC, CTOP + RPC or IPC, CHE + RPC or IPC, GF + RPC or IPC, GLY + RPC or IPC, 5-HD + RPC or IPC, and HMR + RPC or IPC.

AAR = area at risk; LV = left ventricle volume; RPC10, 50, 100 indicate remifentanil concentration in Krebs solution are 10, 50, and 100 ng/ml, respectively; RV = right ventricle volume.

Remifentanil preconditioning or IPC also decreased the release of LDH at 5 or 10 min into reperfusion, and the effects were attenuated by 5×10^{-6} mM naltrindol or 5×10^{-6} mM nor-binaltorphimine but not by 5×10^{-6} mM CTOP (figs. 5A–D).

Heart rate and CF data are summarized in table 2. Coronary artery occlusion resulted in a marked decreased in CF. There was no difference among groups during ischemia and reperfusion in HR and CF.

Effects of RPC or IPC on Myocardial Infarct Size and LDH after Ischemia and Reperfusion with Blockade of PKC or K_{ATP} Channel

Chelerythrine (5×10^{-6} mM) or GF109203X (1×10^{-5} mM), both PKC inhibitors, abolished the effects of RPC or IPC on IS/AAR (figs. 6A and B) and LDH released (figs. 7A–D). Both glibenclamide (1×10^{-5} mM), a non-selective K_{ATP} channel blocker, and 5-hydroxydecanoate (1×10^{-4} mM), a selective mito-K_{ATP} channel blocker,

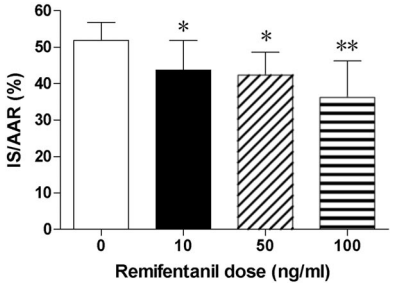

Fig. 2. Effect of remifentanil preconditioning on infarct size, as a percentage of the area at risk (IS/AAR), concentration dependently. IS/AAR in rat hearts subjected to remifentanil 0 (control), 1, 5, and 10 ng/ml. Values are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$ *versus* concentration 0 (control).

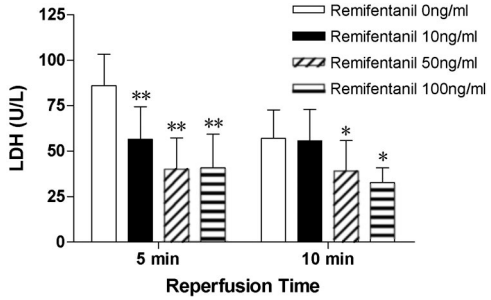

Fig. 3. Effect of remifentanil preconditioning on lactate dehydrogenase (LDH) release at 5 and 10 min after reperfusion. Values are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$ *versus* concentration 0 (control).

Table 2. Hemodynamic Parameters

		Baseline		Treatment		Ischemia		Reperfusion	
Treatment	n	HR	CF	HR	CF	HR	CF	HR	CF
CON	8	299 ± 16	8.6 ± 1.2			290 ± 20	5.3 ± 0.9	235 ± 21	3.9 ± 0.5
RPC (10 ng/kg)	8	301 ± 19	8.0 ± 1.6	294 ± 18*	8.8 ± 1.5†	291 ± 19	4.9 ± 1.7	223 ± 18	4.6 ± 0.9
RPC (50 ng/kg)	8	289 ± 20	8.1 ± 0.3	279 ± 17†	8.8 ± 1.1†	287 ± 18	4.6 ± 1.2	238 ± 20	4.3 ± 1.1
RPC (100 ng/kg)	8	297 ± 17	8.4 ± 1.5	276 ± 16‡	9.6 ± 1.3‡	281 ± 21	4.3 ± 1.7	232 ± 19	3.9 ± 0.2
NTD + RPC	7	284 ± 16	8.4 ± 1.6	272 ± 15‡	7.9 ± 1.1	288 ± 17	4.4 ± 1.7	244 ± 20	4.6 ± 0.8
BNI + RPC	8	281 ± 18	8.0 ± 1.0	269 ± 16‡	7.8 ± 1.2	280 ± 18	4.9 ± 0.9	252 ± 19	3.5 ± 0.5
CTOP + RPC	8	291 ± 18	8.2 ± 1.0	277 ± 17‡	7.8 ± 0.9	299 ± 18	4.5 ± 1.4	241 ± 21	3.0 ± 0.5
CHE + RPC	8	292 ± 17	8.2 ± 1.2	273 ± 16‡	9.1 ± 1.5†	290 ± 17	4.1 ± 0.8	243 ± 19	3.4 ± 0.8
GF + RPC	7	292 ± 16	7.6 ± 1.3	264 ± 17‡	7.3 ± 0.9	289 ± 16	4.8 ± 0.9	232 ± 22	4.1 ± 1.5
GLY + RPC	7	302 ± 17	9.0 ± 1.9	279 ± 18‡	7.4 ± 1.4†	294 ± 18	4.7 ± 1.4	247 ± 19	3.1 ± 0.9
5-HD + RPC	6	281 ± 17	8.7 ± 1.0	270 ± 17‡	8.4 ± 1.1	277 ± 17	4.7 ± 0.5	250 ± 23	4.3 ± 1.0
HMR + RPC	8	293 ± 17	8.3 ± 2.1	270 ± 16‡	6.2 ± 1.9*†	287 ± 18	4.8 ± 2.0	236 ± 23	5.1 ± 1.3
IPC	8	271 ± 17	9.0 ± 0.7	269 ± 16	9.4 ± 1.2	270 ± 17	4.2 ± 1.1	241 ± 21	3.1 ± 0.9
NTD + IPC	7	272 ± 18	7.8 ± 0.4	281 ± 19	7.4 ± 0.6	273 ± 18	3.9 ± 0.8	233 ± 23	4.4 ± 0.5
BNI + IPC	8	293 ± 18	8.7 ± 1.7	289 ± 18	8.0 ± 1.0	290 ± 18	4.1 ± 0.9	251 ± 18	4.2 ± 0.7
CTOP + IPC	8	293 ± 17	7.3 ± 2.0	297 ± 18	7.4 ± 1.2	294 ± 17	4.4 ± 0.9	252 ± 22	4.5 ± 0.4
CHE + IPC	7	291 ± 16	8.2 ± 1.4	285 ± 16	9.4 ± 1.2†	287 ± 17	4.0 ± 1.0	248 ± 19	2.9 ± 0.7
GF + IPC	8	302 ± 18	8.4 ± 1.1	294 ± 18	9.0 ± 0.8	298 ± 19	4.3 ± 1.3	254 ± 19	3.8 ± 0.9
GLY + IPC	8	283 ± 17	8.6 ± 2.2	290 ± 18	7.0 ± 1.3†	283 ± 17	5.2 ± 1.8	242 ± 18	3.3 ± 1.3
5-HD + IPC	7	298 ± 17	7.9 ± 1.3	291 ± 17	8.1 ± 1.1	291 ± 20	4.9 ± 0.7	248 ± 20	5.0 ± 0.7
HMR + IPC	8	282 ± 17	7.6 ± 1.6	289 ± 16	7.9 ± 1.2	278 ± 18	4.7 ± 0.3	232 ± 17	4.1 ± 1.0
NTD	6	271 ± 18	8.9 ± 1.9	279 ± 17	8.0 ± 1.1	268 ± 19	4.1 ± 1.9	234 ± 20	4.6 ± 0.7
BNI	5	290 ± 18	8.3 ± 1.3	282 ± 17	8.1 ± 1.2	291 ± 18	5.2 ± 0.7	239 ± 18	3.4 ± 0.3
CTOP	6	292 ± 17	8.1 ± 1.1	295 ± 18	7.6 ± 1.3	288 ± 17	4.1 ± 0.8	237 ± 19	3.7 ± 1.1
CHE	5	293 ± 16	7.8 ± 0.5	286 ± 17	9.7 ± 1.3†	291 ± 17	3.7 ± 1.0	245 ± 18	3.3 ± 1.3
GF	6	302 ± 18	8.0 ± 1.1	293 ± 18	8.1 ± 1.0	298 ± 18	4.6 ± 0.9	246 ± 21	4.7 ± 0.9
GLY	6	288 ± 19	8.9 ± 0.9	290 ± 18	7.2 ± 0.6†	289 ± 19	4.2 ± 0.6	232 ± 19	3.6 ± 0.7
5-HD	5	293 ± 17	8.4 ± 2.2	296 ± 17	7.8 ± 1.9	293 ± 17	4.4 ± 1.1	293 ± 17	5.3 ± 1.1
HMR	6	286 ± 16	7.9 ± 1.3	291 ± 17	8.1 ± 1.1	286 ± 16	4.9 ± 0.7	286 ± 16	5.0 ± 0.7

Values are presented as mean ± SD. Heart rate (HR) and coronary flow (CF) in rat hearts subjected to CON (control), naltrindol (NTD, 5×10^{-6} M), nor-binaltorphimine (BNI, 5×10^{-6} M), CTOP (1×10^{-6} M), chemerythrine (CHE, 5×10^{-6} M), GF109203X (GF, 1×10^{-6} M), glibenclamide (GLY, 1×10^{-5} M), 5-hydroxydecanoate (5-HD, 1×10^{-4} M), HMR-1098 (HMR, 1×10^{-4} M), remifentanil preconditioning (RPC), ischemic preconditioning (IPC), NTD + RPC or IPC, BNI + RPC or IPC, CTOP + RPC or IPC, CHE + RPC or IPC, GF + RPC or IPC, GLY + RPC or IPC, 5-HD + RPC or IPC, and HMR + RPC or IPC.

* $P < 0.05$ *vs.* RPC (100 ng/ml). † $P < 0.05$, ‡ $P < 0.01$ *vs.* baseline.

Baseline = 15 min after stabilization; ischemia = 30 min after regional ischemia; reperfusion = 2 h after reperfusion; treatment = after RPC.

also abolished the cardioprotection of RPC or IPC (figs. 6A and B and 7A–D). On the other hand, HMR-1098 (1×10^{-4} mM), a selective sarc-K_{ATP} channel blocker, did not do so (fig. 6A and B and 7A–D).

Chelerythrine or HMR-1098 caused a slight but significant increase in CF, whereas glibenclamide led to a decrease in CF. However, there were no differences in CF or HR among all groups during ischemia and reperfusion (table 2).

All three OR antagonists and blockers themselves had no effect on IS/AAR and LDH (figs. 4A, 5A, 5B, 6A, 7A, and 7B).

Discussion

In a previous study, we found that systemic administration of remifentanil, an ultrashort-acting μ-OR agonist, confers cardioprotection, and the effect is attenuated by systemic administration of any one of the three OR subtype antagonists. The observation indicates that blockade of any these OR subtypes located inside or outside the heart abolished cardioprotection of remifentanil.[1] On the other hand, cardioprotection of IPC is abolished by blockade of only κ- or δ-ORs,[1] which is in agreement with the fact that only κ- and δ-ORs are present in the heart.[2,4–7] Because μ-OR is not found in the heart, the μ-OR involved must be located outside the heart. In view of the fact that morphine, a μ-OR agonist, also affects cardiac function *via* both κ- and δ-ORs,[8] we hypothesized that the cardiac κ- and δ-ORs may mediate the cardioprotection of RPC. In the current study, we made use of an isolated perfused heart preparation, which allowed us to determine whether the action of a drug was directly on the heart. We found that direct administration of remifentanil to the heart also reduced myocardial infarct/injury induced subsequently by ischemia and reperfusion, indicating cardioprotection. Interestingly, the cardioprotection was abolished with blockade of κ- or δ- but not μ-ORs. This is unequivocal evidence that RPC also confers cardioprotection *via* cardiac κ- and δ-ORs. In addition, we observed in the current study that the cardioprotection of RPC and IPC share the same signaling mechanisms, namely PKC and mito-K_{ATP} channels. Because activation of cardiac κ- and δ-ORs activates both PKC

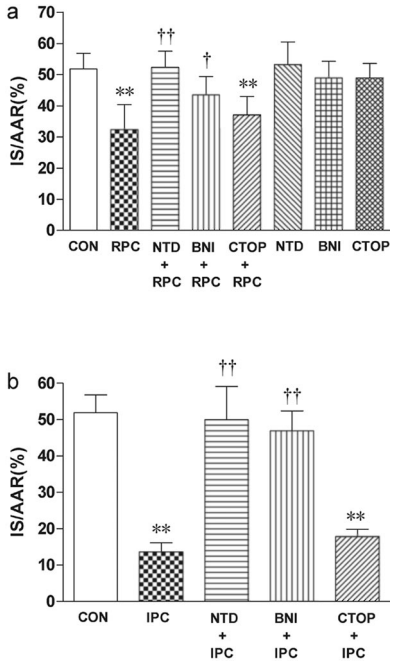

Fig. 4. The effect of opioid antagonists on remifentanil preconditioning (RPC) or ischemic preconditioning (IPC). Infarct size, as a percentage of the area at risk (IS/AAR) in rat hearts subjected to control (CON), naltrindol (NTD, 5×10^{-6} M), nor-binaltorphimine (BNI, 5×10^{-6} M), CTOP (5×10^{-6} M), RPC, IPC, NTD + RPC or IPC, BNI + RPC or IPC, and CTOP + RPC or IPC (*A* and *B*). Values are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$ *versus* control; † $P < 0.05$, †† $P < 0.01$ *versus* RPC or IPC.

and mito-K_{ATP} channels,[10–12,14,16,19–22] the observation provides further support that RPC may involve cardiac κ- and δ-ORs. However, the possibility that remifentanil also acts at these two ORs at other sites, resulting in cardioprotection, cannot be ruled out.

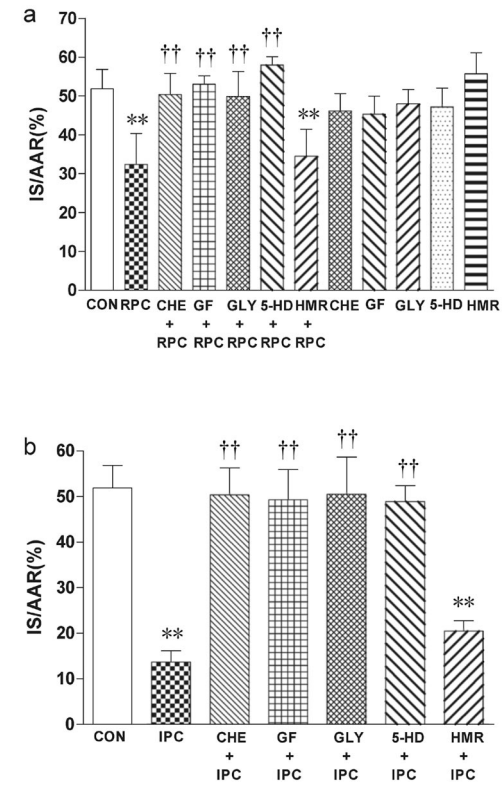

Fig. 6. The effect of protein kinase C (PKC) or adenosine triphosphate–sensitive potassium channel blocker on remifentanil preconditioning (RPC) or ischemic preconditioning (IPC). Infarct size (IS), as a percentage of the area at risk (IS/AAR). Rat hearts subjected to control (CON), chelerythrine (CHE, 5×10^{-6} M), GF109203X (GF, 1×10^{-5} M), glibenclamide (GLY, 1×10^{-5} M), 5-hydroxydecanoate (5-HD, 1×10^{-4} M), HMR-1098 (HMR, 1×10^{-4} M), RPC, IPC, CHE + RPC or IPC, GF + RPC or IPC, GLY + RPC or IPC, 5-HD + RPC or IPC, and HMR + RPC or RPC (*A* and *B*). Values are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$ *versus* control; † $P < 0.05$, †† $P < 0.01$ *versus* RPC or IPC.

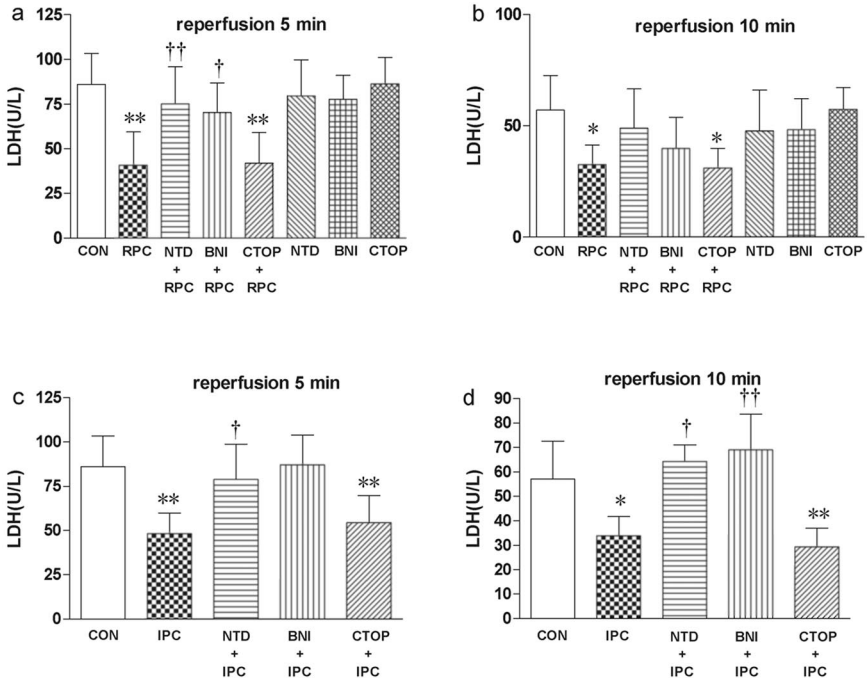

Fig. 5. The effect of opioid antagonists on remifentanil preconditioning (RPC) or ischemic preconditioning (IPC). Lactate dehydrogenase (LDH) release in rat hearts subjected to control (CON), naltrindol (NTD, 5×10^{-6} M), nor-binaltorphimine (BNI, 5×10^{-6} M), CTOP (5×10^{-6} M), RPC, IPC, NTD + RPC or IPC, BNI + RPC or IPC, and CTOP + RPC or IPC (*A–D*). Values are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$ *versus* control; † $P < 0.05$, †† $P < 0.01$ *versus* RPC or IPC.

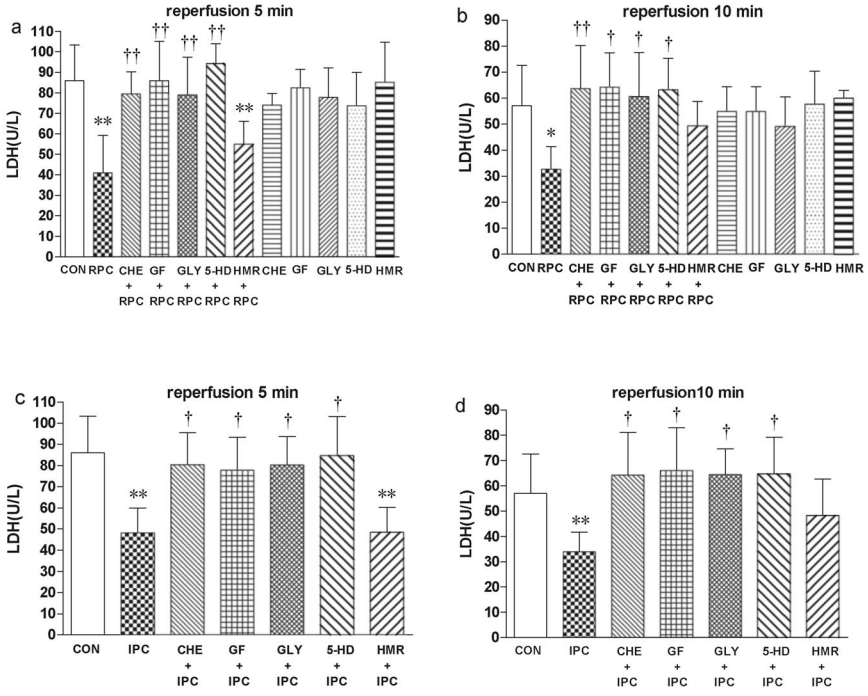

Fig. 7. The effect of protein kinase C (PKC) or adenosine triphosphate–sensitive potassium channel blocker on remifentanil preconditioning (RPC) or ischemic preconditioning (IPC). Lactate dehydrogenase (LDH) release in rat hearts subjected to control (CON), chelerythrine (CHE, 5×10^{-6} M), GF109203X (GF, 1×10^{-5} M), glibenclamide (GLY, 1×10^{-5} M), 5-hydroxydecanoate (5-HD, 1×10^{-4} M), HMR-1098 (HMR, 1×10^{-4} M) (*A* and *B*), RPC, IPC, CHE + RPC or IPC, GF + RPC or IPC, GLY + RPC or IPC, 5-HD + RPC or IPC, and HMR + RPC or RPC (*A–D*). Values are presented as mean ± SD. * $P < 0.05$, ** $P < 0.01$ *versus* control; † $P < 0.05$, †† $P < 0.01$ *versus* RPC or IPC.

In our previous study, we found that the cardioprotection of systemic administration of remifentanil is mediated *via* all three types of ORs, whereas that of IPC is mediated only *via* κ- and δ-ORs.[1] This observation suggested that only extracardiac μ-OR is involved in the cardioprotection of RPC. In support of this finding, the current study showed that cardioprotection of remifentanil directly administered to the isolated perfused heart was not blocked by μ-OR blockade. This concurs with the fact that μ-OR is not present in the heart.

Our data showed that the PKC inhibitors, chelerythrine and GF109203X, abolished the protective effect of RPC, as in the case of IPC. This is in agreement with Miki *et al.*,[21] who found that the cardioprotective effect of morphine could be blocked by chelerythrine. The finding is also in agreement with the finding of Kato *et al.*,[23] who showed that fentanyl limits infarction by PKC activation. More importantly, this is consistent with the fact that the cardioprotection of activation of cardiac δ- or κ-ORs involves PKC.[12,14,21] Our results provide further support that the cardioprotection effect of RPC is mediated *via* cardiac δ- and κ-ORs.

Our data also help to determine the role of the sarc-K_{ATP} channel and the mito-K_{ATP} channel in mediating the cardioprotection produced by RPC. Although glibenclamide, a nonselective K_{ATP} channel blocker, abolished the effect, HMR-1098, a selective sarc-K_{ATP} blocker, did not block the beneficial effects of remifentanil. However, treatment with 5-hydroxydecanoate, the selective mito-K_{ATP} blocker, abolished remifentanil-induced cardioprotection. These data clearly suggest that RPC is mediated *via* the mito-K_{ATP} channel in the isolated rat heart. Previous studies have also shown that morphine and fentanyl mimic IPC *via* the mito-K_{ATP} channel in myocytes and in the intact heart.[22,24–27]

The remifentanil concentrations we used (10, 50, and 100 ng/ml) were higher than those used in normal human clinical practice, where a range of 5–20 ng/ml would be reasonable if remifentanil was being administered by target-controlled infusion. However, the pharmaceutical data sheet actually recommends an upper dose limit of 2 $\mu g \cdot kg^{-1} \cdot min^{-1}$, which is likely to result in blood concentrations of approximately 50 ng/ml.[28] Nevertheless, it is difficult to extrapolate animal findings to humans, and plasma concentrations obtained are difficult to compare because the pharmacokinetics (*e.g.*, plasma protein binding) may vary considerably between species. From the clinical perspective, however, remifentanil is an interesting drug in that it could easily be given in relatively high doses for a short period of time. It is very titratable, with a short time to peak effect and a very short context-sensitive half-life, ensuring a rapid offset, independent of organ function.

In conclusion, the current study provides unequivocal evidence that κ- and δ- but not μ-ORs mediate the cardioprotection produced by RPC. It does not rule out the possibility that κ- and δ-ORs in extracardiac sites may also mediate the cardioprotection of RPC.

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