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Remifentanil post-conditioning attenuates cardiac ischemia–reperfusion injury via κ or δ opioid receptor activation

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Background: Ischemic pre- or post-conditioning of the heart has been shown to involve opioid receptors. Remifentanil, an ultra-short-acting selective μ opioid receptor agonist in clinical use, pre-conditions the rat heart against ischemia–reperfusion injury. This study investigates whether remifentanil post-conditioning is also cardioprotective.
Methods: Remifentanil post-conditioning (5-min infusion at 1.5, 10–20 μg/kg/min) or ischemic post-conditioning (three cycles of a 10 s reperfusion interspersed with a 10 s ischemia) was induced in an open-chest rat heart model of ischemia and reperfusion injury, in the presence or absence of nor-binaltorphimine, naltrindole or CTOP, specific κ, δ and μ opioid receptor antagonists, respectively. The same sequence of experiments was repeated in the isolated heart model using the maximal protective dose of remifentanil from the dose–response studies.
Results: Both ischemic and remifentanil post-conditioning reduced the myocardial infarct size relative to the control group in both models. This cardioprotective effect for both post-conditioning regimes was prevented by the prior administration of nor-binaltorphimine and naltrindole but not CTOP. The sole administration of the antagonists had no effect on the size of myocardial infarction.
Conclusions: These results indicate that remifentanil post-conditioning protects the heart from ischemia–reperfusion injury to a similar extent as of ischemic post-conditioning. This protection involves κ and δ but not μ opioid receptor activation. This drug has great potential as a clinical post-conditioning modality as it can be given in large doses without prolonged opioid-related side effects.

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CARDIAC post-conditioning refers to therapeutic maneuvers administered just before final reperfusion that attenuate ischemia–reperfusion injury. Ischemic post-conditioning involving staccato reperfusion reduces infarct size (IS) to an extent comparable to that achieved by pre-conditioning,[1] and molecular studies have implicated several common components and pathways.[2] Opioid receptors are involved in ischemic post-conditioning, as the latter can be blocked by the peripherally restricted opioid antagonist naloxone methiodide[3] and the δ-specific antagonist naltrindole (NTD).[4] Not until recently has the role of μ receptors in post-conditioning been specifically addressed[5] as it has traditionally been thought to be absent from the heart,[6] although more recent binding studies have challenged this.[7]

Remifentanil, a selective μ agonist, pre-conditions the heart in the intact rat in part via μ receptor activation, possibly in a location outside the heart.[8,9] As common reperfusion injury salvage pathways may be triggered by pre- and post-conditioning,[10] remifentanil could potentially post-condition the myocardium. This study, evaluates whether remifentanil is cardioprotective when administered in a post-conditioning fashion and compares its effect with that of ischemic post-conditioning. The relative role of opioid receptor subtypes in both regimes was also investigated by the use of subtype-specific opioid receptor antagonists.

Material and methods

All procedures were approved by the local Committee for the use of live animals in teaching and research. Experiments were conducted using 8-week-old male Sprague–Dawley rats weighing

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300 ± 25 g, which were housed in separate cages, given free access to food and water, except before the study, and were exposed to alternate 12-h light and dark cycles. A total of 114 animals were used for in the *vivo* and 74 for the isolated heart experiments.

In vivo induction of ischemia–reperfusion injury

An anesthetized open-chest model of ischemia and reperfusion injury was used. The anesthetic and surgical preparation to the point of post-conditioning and the IS determination have been described in detail previously.[11] In short, anesthesia was induced using pentobarbitone (50 mg/kg) and maintained with boluses of 25 mg/kg 90 min after induction. The heart was exposed via left thoracotomy at the fifth intercostal space. Repeated cycles of regional ischemia and reperfusion were made by tightening or releasing the snare placed at the origin of the left coronary artery. More prolonged ischemia involved securing the sutures with a mosquito hemostat. Ischemia was confirmed by cardiac cyanosis, a substantial decrease in the mean arterial pressure and electrocardiographic changes.

Isolated rat heart preparation

After the removal from the anesthetized rat, the heart was immediately perfused by the Langendorff method, and subsequently converted to the working heart model (preload 15 cmH_2O, afterload 80 cmH_2O). Modified Krebs–Henseleit bicarbonate buffer was used as the perfusion buffer (K–H buffer, mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, KH_2PO_4 1.2, EDTA 0.5, $NaHCO_3$ 25, glucose 11, pH 7.4, 37 °C, 95% O_2+5% CO_2 gas mixture). Electrocardiograms and indices of left ventricular performance pressure [left ventricular developed pressure (LVDP), left ventricular end diastolic pressure (LVEDP), positive and negative maximum left ventricular pressure derivative ($+dP/dt$ and $-dP/dt$)] were measured using a Power-Lab monitoring system with a Mikro-Tip Pressure Catheter (AD Instruments, Colorado Springs, CO). After an initial stabilization period of 15 min, ligation of the left coronary artery was performed using a 6-0 prolene loop, along with a snare occluder, to mimic a regional ischemia condition for 30 min, followed by 120 min of reperfusion.

Myocardial IS determination

After the 120 min of reperfusion, the hearts from the *in vivo* were excised and transferred to a Langendorff apparatus. Each heart was immediately perfused with normal saline for 1 min at a pressure of 100 cmH_2O to remove residual blood. The left coronary artery was re-occluded and 0.25% Evans blue dye was injected to stain the normally perfused region of the heart. Evans blue negative area represented the area at risk (AAR) from occlusion of the left coronary artery. The hearts were then frozen, cut into 2 mm slices, incubated at 37 °C for 20 min in 1% 2, 3, 5-triphenyltetrazolium (Sigma Chemical Company, St Louis, MO) in phosphate buffer at pH 7.4 and then immersed in 10% formalin for 20 min to enhance the contrast of the stain. The areas of infarct (triphenyltetrazolium negative) and the risk zone for each slice were traced and digitized using a computerized-planimetry technique (SigmaScan 4.0, Systat Software Inc., Richmond, CA). The volumes of the left ventricles, IS and AAR were calculated by multiplying area with slice thickness and summing the product. The IS was expressed as a percentage of the AAR (IS/AAR), and this ratio was used to compare the differences between the groups.

Treatment protocols (Fig. 1)

Intact animal studies. All animals were subjected to 30 min of ischemia, followed by 120 min of reperfusion. Rats were omitted from further data analysis if severe hypotension (arterial mean blood pressure <30 mmHg) or intractable ventricular fibrillation occurred. At 5 min before the onset of reperfusion, the animals were allocated to different treatments according to a predetermined randomized sequence. All the drugs used were dissolved in normal saline for administration.

Dose–response studies. For a negative control group, normal saline was infused for a period of 5 min beginning just before reperfusion. Remifentanil post-conditioning was evaluated using a 5-min infusion of the drug at 1, 5, 10 or 20 μg/kg/min of body weight (GlaxoSmithKline Limited, Hong Kong, ~~Hong Kong~~). In order to achieve near-steady-state levels at reperfusion, the infusion was commenced 5 min before the release of the snare occluder. For a positive control, ischemic post-conditioning was used and comprised of three cycles of 10 s of reperfusion and 10 s of ischemia before the final reperfusion.

This regime was chosen based on previous studies in a rat model that was shown to be effective.[12,13] The remifentanil dose at which max-

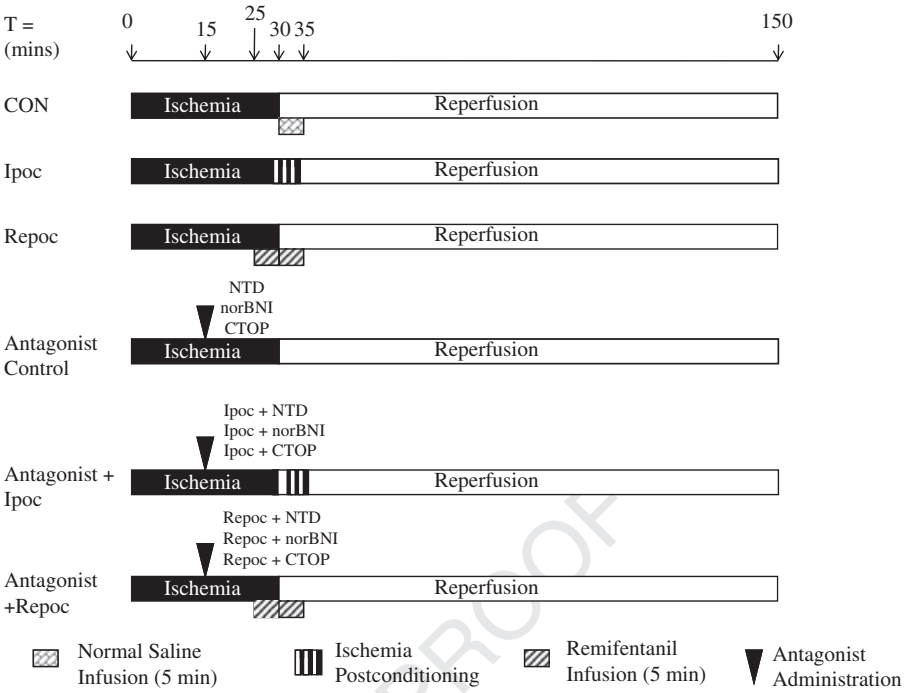

Fig. 1. Study design for in vivo *and isolated heart preparation experiments. Ischemia–reperfusion injury was induced by 30 min of left coronary artery ligation, followed by 120 min of reperfusion. CON, control; Ipoc, ischemic post-conditioning (three cycles of 10 s of ischemia alternating with 10 s of reperfusion); Repoc, remifentanil post-conditioning (1, 5, 10 or 20 μg/kg/min); NTD, naltrindole; nor BNI, nor-binaltorphimine and CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2.*

imal protection occurred was selected for the antagonists and isolated heart studies.

Antagonist studies. Each of the antagonists was given 15 min before reperfusion to evaluate any intrinsic effects they may have had on myocardial IS. These compounds were NTD, a δ opioid receptor selective antagonist,[14] D-Phe–Cys–Tyr-D-Trp–Orn–Thr–Pen–Thr–NH_2 (CTOP), a μ opioid receptor selective antagonist,[15] and nor-binaltorphimine (nor-BNI), a κ opioid receptor selective antagonist[16] (Sigma Chemical Company). These selective opioid receptor antagonists were dissolved in normal saline and administered as a bolus at the following doses: NTD (5 mg/kg); nor BNI (5 mg/kg) and CTOP (1 mg/kg). Both ischemic post-conditioning and remifentanil post-conditioning (20 μg/kg/min) were then performed in the presence of individual antagonists administered 15 min before reperfusion.

Isolated heart studies. The same sequences of experiments were performed in the isolated heart subjected to simulated ischemia and reperfusion. Only the dose of remifentanil that produced the maximal reduction of IS in the intact animal was used in the isolated heart study. Although it is unlikely that remifentanil will reach the ischemic myocardium, it was introduced 5 min before reperfusion to mimic the *in vivo* preparation and continued for 5 min after the release of the snare occlude.

Statistical analysis

The primary outcome is myocardial IS, expressed as percentage of the area at risk (IS/AAR). Previous data from our laboratory using this model of cardiac ischemia–reperfusion injury indicated the expected IS/AAR of the control group to be between 50% and 60% and the expected magnitude of IS/AAR reduction to be 40–50%. Therefore, at least five animals per group are required to yield a power of 80% and a *P*-value of 0.05. All data are expressed as mean ± SD, and were obtained from six to seven separate animals per group. Statistical significance was determined by one-way analysis of variance (ANOVA), with application of Bonferroni correction if significant *F* ratios were obtained. Hemodynamic data were analyzed using one-way ANOVA for between-group comparisons and repeated measure ANOVA for comparisons between time points (SPSS version 16.0 for windows).

Results

A total of 114 animals completed the *in vivo* experiments. Nineteen rats were excluded from further analysis as they developed refractory hypotension ($n = 4$) and ventricular fibrillation ($n = 15$) during the induction of regional ischemia. They have yet received any experimental drugs. A total of 74 rats were used for the isolated heart preparations.

In vivo hemodynamic data

The hemodynamic data for the dose–response studies are presented in Table 1, and those for the antagonist experiments are presented in Table 2. Hemodynamic values including heart rate (HR), mean arterial blood pressure (MAP) and rate–pressure product (RPP) did not differ between groups ($P > 0.05$) at baseline at the end of the ischemic or reperfusion periods for both series of experiments. For the dose–response experiments, remifentanil post-conditioning reduced the HR and RPP, except for the 10 μg/kg dose. Both the 5 and the 20 μg/kg/min dose reduced the MAP. In the antagonist experiments, the HR and RPP in all the groups were also significantly lower during post-conditioning compared with the control group, with the MAP reduced only in the remifentanil-containing groups.

In vivo IS comparisons

The AAR ranged from 0.36 ± 0.02 to $0.44 \pm 0.03\,cm^3$ and there were no significant differences between the treatment groups. The IS/AAR was reduced by remifentanil post-conditioning at doses of 10 μg/kg/min ($40 \pm 4\%$) and 20 μg/kg/min ($39 \pm 6\%$), as well as ischemic post-conditioning ($40 \pm 6\%$) when compared with the control group ($55 \pm 7\%$) ($P < 0.05$) (Fig. 2). Although there was a reduction in IS/AAR using 5 μg/kg/min ($45. \pm 6\%$), it did not reach statistical significance when compared with the control ($P = 0.07$). However, there was no difference in the infarct-sparing effect between the two modes of post-conditioning ($P = 1.0$). The addition of NTD or nor-BNI before both ischemic and remifentanil pre-conditioning prevented their protective effects. However, CTOP had no significant effect on either post-conditioning regime. The sole administration of individual opioid receptor antagonists did not change the IS compared with the control (Fig. 3).

Hemodynamic indices in the isolated heart

The HR and indices of left ventricular performance are presented in Table 3. There were no differences between groups at baseline, during ischemia, at 60 and 120 min after reperfusion for all indices. There were also no differences between groups for the positive and negative dp/dt values for all time points. Remifentanil post-conditioning reduced the LVDP, LVEDP and HR at 10 min after reperfusion. Repoc+nor-BNI reduced LVDP and HR at 10 min after reperfusion, whereas Repoc+NTD and

Table 1

Hemodynamic data of the dose response studies.

	n	Baseline	Ischemia	Post-conditioning	Reperfusion
MAP (mmHg)					
CON	6	99 ± 11	96 ± 10	93 ± 10	77 ± 16*
IPOC	6	107 ± 9	102 ± 11	81 ± 3*	90 ± 10*
Repoc 1	6	121 ± 21	80 ± 16*	71 ± 20*	93 ± 15
Repoc 5	6	107 ± 12	77 ± 8*	63 ± 18*,†	83 ± 18
Repoc 10	6	122 ± 24	93 ± 19*	73 ± 22*	102 ± 19
Repoc 20	7	103 ± 5	97 ± 11	65 ± 8*,†	88 ± 11
HR (per minute)					
CON	6	423 ± 24	406 ± 12	413 ± 18	368 ± 21*
IPOC	6	423 ± 21	416 ± 19	373 ± 14*	390 ± 14*
Repoc 1	6	378 ± 46	377 ± 44	323 ± 40†	341 ± 39
Repoc 5	6	392 ± 34	396 ± 25	353 ± 50†	325 ± 23
Repoc 10	6	380 ± 48	382 ± 57	370 ± 31	366 ± 52
Repoc 20	7	417 ± 21	408 ± 16	345 ± 14*,†	385 ± 19
RPP (mmHg/min/1000)					
CON	6	42 ± 4	39 ± 4	38 ± 4	28 ± 6*
IPOC	6	45 ± 4	43 ± 3	30 ± 2*	34 ± 4*
Repoc 1	6	45 ± 9	30 ± 6*	23 ± 8*,†	32 ± 7*
Repoc 5	6	42 ± 4	31 ± 5*	23 ± 9*,†	27 ± 7*
Repoc 10	6	46 ± 9	36 ± 10*	27 ± 10*	38 ± 11
Repoc 20	7	42 ± 4	39 ± 5*	22 ± 4*,†	34 ± 5*

Data were collected at the end of the respective periods and are presented as mean ± SD; data are compared against baseline value within-group using a repeated measure analysis of variance (ANOVA) and between groups are made using one-way ANOVA, with the Bonferroni correction applied for multiple comparisons if significant *F* ratios were obtained.
*$P < 0.05$ vs. baseline (within-group comparison).
†$P < 0.05$ vs. control (between-group comparison).
MAP, mean arterial pressure; HR, heart rate; RPP, rate pressure product; CON, control group; Repoc, remifentanil post-conditioning.

Table 2

Hemodynamic data antagonist *in vivo* experiments.

	n	Baseline	End of ischemia period	End of post-conditioning period	End of reperfusion period
MAP (mmHg)					
CON	6	99 ± 11	96 ± 10	93 ± 10	77 ± 16*
NTD	6	102 ± 11	101 ± 10	82 ± 14	83 ± 17
nor-BNI	7	102 ± 13	102 ± 16	77 ± 12*	79 ± 14*
CTOP	7	101 ± 13	99 ± 12	80 ± 16	81 ± 13
Ipoc+NTD	6	111 ± 13	105 ± 14	74 ± 14*	77 ± 16*
Ipoc+nor-BNI	7	107 ± 13	106 ± 13	77 ± 10*	82 ± 16*
Ipoc+CTOP	6	102 ± 16	102 ± 11	81 ± 14	81 ± 19
Repoc+NTD	6	98 ± 14	97 ± 13	64 ± 12†	86 ± 16
Repoc+nor-BNI	6	101 ± 8	100 ± 9	69 ± 11*,†	86 ± 13
Repoc+CTOP	7	116 ± 10	110 ± 13	67 ± 14*,†	89 ± 15*
HR (beats per minute)					
CON	6	423 ± 24	406 ± 12	413 ± 18	368 ± 21*
NTD	6	415 ± 22	411 ± 23	373 ± 19†	381 ± 14
nor-BNI	7	413 ± 23	406 ± 17	377 ± 21†	379 ± 20
CTOP	7	430 ± 15	412 ± 23	374 ± 20*,†	378 ± 21*
Ipoc+NTD	6	421 ± 23	411 ± 19	377 ± 13*,†	379 ± 16*
Ipoc+nor-BNI	7	414 ± 14	414 ± 19	380 ± 21*,†	375 ± 18*
Ipoc+CTOP	6	415 ± 14	411 ± 14	383 ± 13*,†	384 ± 15
Repoc+NTD	6	410 ± 29	408 ± 24	371 ± 18*,†	382 ± 23
Repoc+nor-BNI	6	421 ± 21	422 ± 20	362 ± 5*,†	381 ± 20
Repoc+CTOP	7	420 ± 22	409 ± 28	367 ± 15*,†	387 ± 19
RPP (mmHg/min/1000)					
CON	6	42 ± 4	42 ± 4	38 ± 4	28 ± 6*
NTD	6	43 ± 6	43 ± 6	31 ± 5†	32 ± 7
nor-BNI	7	42 ± 7	42 ± 7	29 ± 6*,†	30 ± 6*
CTOP	7	43 ± 6	43 ± 6	30 ± 7*,†	31 ± 6*
Ipoc+NTD	6	47 ± 5	47 ± 5	28 ± 5*,†	30 ± 5*
Ipoc+nor-BNI	7	44 ± 7	44 ± 7	29 ± 3*,†	30 ± 5*
Ipoc+CTOP	6	43 ± 8	43 ± 8	31 ± 6*,†	31 ± 8*
Repoc+NTD	6	40 ± 9	40 ± 9	24 ± 11*,†	33 ± 10*
Repoc+nor-BNI	6	43 ± 5	43 ± 5	25 ± 4*,†	33 ± 6*
Repoc+CTOP	7	48 ± 4	48 ± 4	25 ± 5*,†	35 ± 6*

Data are presented as mean ± SD; data are compared against baseline value across different time points using a repeated measure analysis of variance (ANOVA) and between groups using one-way ANOVA, with the Bonferroni correction applied for multiple comparisons if significant *F* ratios were obtained.
*$P<0.05$ vs. baseline (within-group comparison).
†$P<0.05$ vs. control (between-group comparison).
MAP, mean arterial pressure; HR, heart rate; CON, control group; Ipoc, ischemic post-conditioning; Repoc, remifentanil post-conditioning; NTD, naltrindole; nor-BNI, nor-binaltorphimine; CTOP, D-Phe–Cys–Tyr–D-Trp–Orn–Thr–Pen–Thr–NH_2.

Repoc+CTOP also reduced LVDP at the same time point.

Isolated heart IS comparisons

The AAR ranged from 0.38 ± 0.07 to $0.55 \pm 0.06\,cm^3$. The IS/AAR for both ischemic post-conditioning ($44 \pm 5\%$) and remifentanil post-conditioning ($42 \pm 4\%$) were significantly smaller relative to the control group ($59.0 \pm 3\%$) ($P<0.01$). However, there was no difference in the infarct-sparing effect between the two modes of post-conditioning ($P=0.38$). Similar to the *in vivo* data, the addition of NTD or nor-BNI before both ischemic and remifentanil pre-conditioning prevented their protective effects. The addition of CTOP also had no significant effect on either post-conditioning regime. The sole administration of individual opioid receptor antagonists did not change the IS compared with the control (Fig. 4).

Discussion

The results of this study have demonstrated that the application of an exogenous opioid in the form of remifentanil after the start of the ischemic event diminishes cardiac ischemia–reperfusion injury to an extent similar to that from ischemic post-conditioning, using both the intact rat and the isolated heart perfusion model. There is an indication that the degree of protection is related to the dose

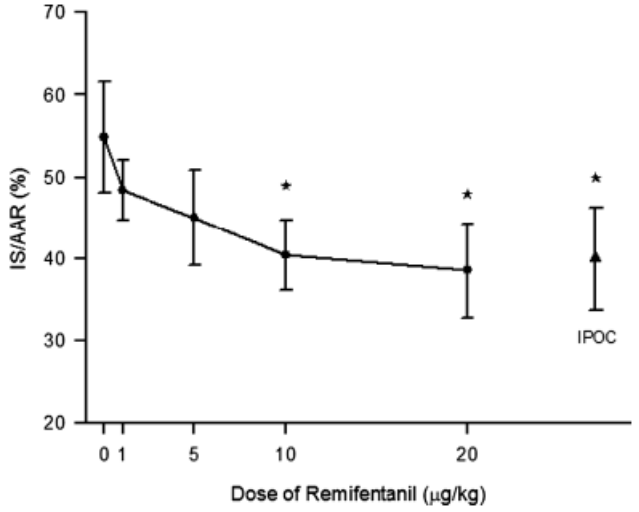

Fig. 2. Graph showing infarct size (IS) as a percentage of the area at risk (AAR) for increasing remifentanil dose. The effect of ischemic post-conditioning (Ipoc) is also shown for comparison. Results are plotted as mean ± standard deviation. *$P < 0.05$.

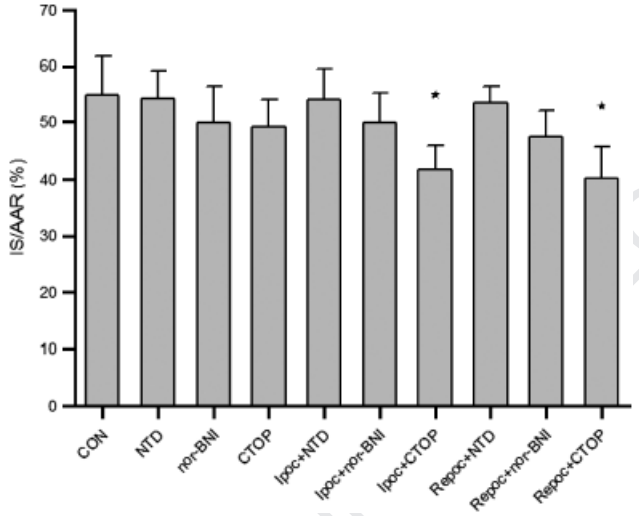

Fig. 3. Comparison of the infarct size (IS) as a percentage of the area at risk (AAR) for the different treatment groups in vivo. *Error bars = ± standard deviations. CON, control group; Ipoc, ischemic post-conditioning; Repoc, remifentanil post-conditioning; NTD, naltrindole; nor BNI, nor-binaltorphimine; CTOP, D-Phe–Cys–Tyr-D-Trp–Orn–Thr–Pen–Thr–NH_2.* *$P < 0.05$ *vs. control.*

administered in the *in vivo* model. Activation of either δ or κ opioid receptors is necessary for both forms of post-conditioning in this model. The μ receptor appears not to be involved in this process. The efficacy of post-conditioning in the isolated heart model suggests that this process is at least in part locally mediated.

Because post-conditioning was first described with the application of intermittent ischemia, a number of ligand mediators/triggers have now been identified, including adenosine,[12,17] bradykinin[18] and opioids,[19] as well as reactive oxygen species.[18] Although chemically diverse, a common theme underlying these compounds is that they are all increased during ischemia and reperfusion.[20–23] Indeed, some have postulated that ischemic post-conditioning is another form of staged or controlled reperfusion,[24] possibly by altering the levels of these compounds and maintenance of an acidic pH.[17] Increased expression of endogenous opioids in heart tissue around the time of myocardial infarction has long been recognized[25] and activation of opioid receptor subtypes may enhance ischemic tolerance.[26] Activation of δ opioid receptors by morphine has been demonstrated to inhibit the mitochondria permeability transition pore,[4] the putative mechanism for ischemic tolerance and, therefore, opioid post-conditioning. The significance of this study lies is not so much the demonstration of post-conditioning by an exogenous opioid *per se*, but in the fact that the agent is a selective μ opioid receptor agonist in clinical use and its potential clinical significance. The unique pharmacokinetic properties of remifentanil among the opioids would enable rapid attainment of high plasma concentrations without the concern of prolonged opioid-related side effects. Post-conditioning has a small window of effectiveness and rapid achievement of sufficient plasma concentration may not be attainable by other opioids with longer half-lives without resorting to using high doses. This may result in prolonged sedation and/or respiratory depression. Another point of significance on an experimental level is that remifentanil is a selective μ receptor agonist. With the exception of one study,[5] this receptor subtype has not been implicated to be involved in post-conditioning.

In contrast to our current results with post-conditioning, remifentanil mediates its pre-conditioning cardioprotective effect in part via μ receptors in the intact rat,[8] but not in isolated rat heart preparations.[9] Intrathecal morphine at a fraction of the intravenous dose can also pre-condition the heart,[27] an effect attenuated by intrathecal administration of the μ-specific antagonist CTOP.[28] These observations support a role for the activation of extra-cardiac μ receptors in remifentanil pre-conditioning. Such remote pre-conditioning has been demonstrated with other triggers such as ischemia, where pre-conditioning of one organ may confer benefits in a remote organ.[29] However, whether post-conditioning, and in particular opioid post-conditioning, can be remotely triggered remains to be defined. Recent work has suggested that post-

Table 3

Indices of Myocardial Performance of the isolated heart preparations.

	n	Baseline	Ischemia (30 min)	Rep (10 min)	Rep (60 min)	Rep (120 min)
LVDP (mmHg)						
Con	6	100 ± 13	72 ± 12	91 ± 14	84 ± 21	73 ± 17*
NTD	6	97 ± 8	65 ± 10*	87 ± 7*	71 ± 6*	60 ± 6*
nor-BNI	6	93 ± 9	62 ± 6*	83 ± 9	69 ± 6*	59 ± 7*
CTOP	6	96 ± 11	65 ± 9*	85 ± 15	69 ± 7*	63 ± 4*
Ipoc+NTD	6	98 ± 7	67 ± 7*	80 ± 7*	69 ± 7*	62 ± 6*
Ipoc+nor-BNI	6	100 ± 15	62 ± 11*	87 ± 3	72 ± 11*	60 ± 7*
Ipoc+CTOP	6	112 ± 11	74 ± 14*	83 ± 15*	71 ± 10*	62 ± 9*
Repoc+NTD	6	106 ± 19	72 ± 7*	69 ± 5*,†	73 ± 9*	57 ± 7*
Repoc+nor-BNI	6	105 ± 18	67 ± 20*	70 ± 4*,†	75 ± 22*	67 ± 18*
Repoc+CTOP	6	111 ± 23	70 ± 11*	72 ± 9†	66 ± 10	64 ± 5*
LVEDP (mmHg)						
Con	6	6 ± 2	9 ± 1	34 ± 5*	21 ± 7*	20 ± 9
NTD	6	6 ± 1	8 ± 2	28 ± 5*	21 ± 5*	16 ± 5*
nor-BNI	6	6 ± 1	9 ± 1*	30 ± 2*	23 ± 5*	16 ± 4*
CTOP	6	7 ± 1	10 ± 2	31 ± 7*	21 ± 5*	17 ± 5*
Ipoc+NTD	6	6 ± 1	8 ± 4	28 ± 6*	20 ± 5*	15 ± 4*
Ipoc+nor-BNI	6	7 ± 2	12 ± 4*	29 ± 7*	21 ± 5*	18 ± 5
Ipoc+CTOP	6	7 ± 1	8 ± 1	28 ± 4*	18 ± 5*	18 ± 7
Repoc+NTD	6	6 ± 1	8 ± 2	29 ± 6*	20 ± 4*	15 ± 5*
Repoc+nor-BNI	6	5 ± 1	10 ± 2*	26 ± 4*	22 ± 7*	15 ± 3*
Repoc+CTOP	6	5 ± 1	8 ± 3	28 ± 2*	22 ± 6*	17 ± 4*
HR (beats per minute)						
Con	6	240 ± 37	251 ± 22	265 ± 36	231 ± 50	204 ± 57
NTD	6	259 ± 10	255 ± 33	256 ± 31	232 ± 26	207 ± 30
nor-BNI	6	253 ± 45	245 ± 28	231 ± 31	249 ± 37	216 ± 47
CTOP	6	252 ± 25	267 ± 38	239 ± 33	235 ± 39	199 ± 36
Ipoc+NTD	6	253 ± 24	264 ± 21	233 ± 36	211 ± 31	196 ± 19*
Ipoc+nor-BNI	6	266 ± 28	262 ± 38	241 ± 26	213 ± 30	205 ± 33
Ipoc+CTOP	6	241 ± 24	247 ± 25	226 ± 36	219 ± 42	207 ± 46
Repoc+NTD	6	237 ± 31	249 ± 35	207 ± 48	225 ± 62	194 ± 28
Repoc+nor-BNI	6	246 ± 28	262 ± 25	199 ± 22†	219 ± 16	202 ± 33
Repoc+CTOP	6	261 ± 27	255 ± 22	209 ± 16*	237 ± 30	223 ± 37
d*p*/d*t* (mmHg/s)						
Con	6	1952 ± 178	1465 ± 156*	1353 ± 165*	1199 ± 75*	1077 ± 69*
NTD	6	1948 ± 393	1572 ± 285*	1343 ± 243*	1272 ± 197*	1153 ± 160*
nor-BNI	6	2092 ± 311	1591 ± 256*	1334 ± 117*	1210 ± 86*	1083 ± 63*
CTOP	6	2009 ± 349	1487 ± 342*	1280 ± 193*	1182 ± 172*	1058 ± 101*
Ipoc+NTD	6	1989 ± 333	1556 ± 329	1351 ± 267*	1227 ± 236*	1153 ± 211*
Ipoc+nor-BNI	6	2035 ± 216	1676 ± 305*	1439 ± 174*	1246 ± 131*	1150 ± 109*
Ipoc+CTOP	6	2001 ± 275	1549 ± 366	1292 ± 289*	1145 ± 205*	1052 ± 155*
Repoc+NTD	6	2117 ± 235	1673 ± 220*	1243 ± 95*	1181 ± 90*	1114 ± 77*
Repoc+nor-BNI	6	1931 ± 385	1522 ± 330*	1246 ± 156*	1164 ± 165*	1111 ± 131*
Repoc+CTOP	6	2014 ± 373	1550 ± 274*	1226 ± 105*	1156 ± 90*	1102 ± 90*
− d*p*/d*t* (mmHg/s)						
Con	6	1601 ± 342	1363 ± 217	1178 ± 123	1061 ± 70	960 ± 83
NTD	6	1447 ± 193	1272 ± 172*	1168 ± 112*	1062 ± 103*	989 ± 71*
nor-BNI	6	1559 ± 204	1316 ± 140*	1241 ± 173*	1122 ± 124*	997 ± 61*
CTOP	6	1541 ± 348	1345 ± 240	1245 ± 185	1105 ± 149*	1008 ± 98*
Ipoc+NTD	6	1516 ± 174	1217 ± 93	1164 ± 32	1068 ± 85*	990 ± 33*
Ipoc+nor-BNI	6	1548 ± 175	1222 ± 116	1082 ± 59*	987 ± 48*	954 ± 27
Ipoc+CTOP	6	1560 ± 292	1228 ± 130	1123 ± 132	1058 ± 112*	969 ± 97*
Repoc+NTD	6	1676 ± 334	1302 ± 174*	1175 ± 164*	1062 ± 160*	975 ± 140*
Repoc+nor-BNI	6	1533 ± 212	1208 ± 63*	1107 ± 68*	1047 ± 98*	981 ± 91*
Repoc+CTOP	6	1617 ± 235	1344 ± 273	1148 ± 112	1064 ± 86*	1012 ± 52*

Data are presented as mean ± SD; data are compared against baseline value across different time points using a repeated measure analysis of variance (ANOVA) and between groups using one-way ANOVA, with the Bonferroni correction applied for multiple comparisons if significant *F* ratios were obtained. Baseline values obtained just before induction of ischemia.

*$P < 0.05$ vs. baseline (within-group comparison).

†$P < 0.05$ vs. control (between-group comparison).

LVDP, left ventricular developed pressure (mmHg); LVEDP, left ventricular end diastolic pressure; HR, heart rate; d*P*/d*t*, positive left ventricular pressure derivative (mmHg/s); − d*P*/d*t*, negative left ventricular pressure derivative (mmHg/s); CON, control group; Ipoc, ischemic postconditioning; Repoc, remifentanil post-conditioning; NTD, naltrindole; nor-BNI, nor-binaltorphimine; CTOP, D-Phe–Cys–Tyr-D-Trp–Orn–Thr–Pen–Thr–NH_2.

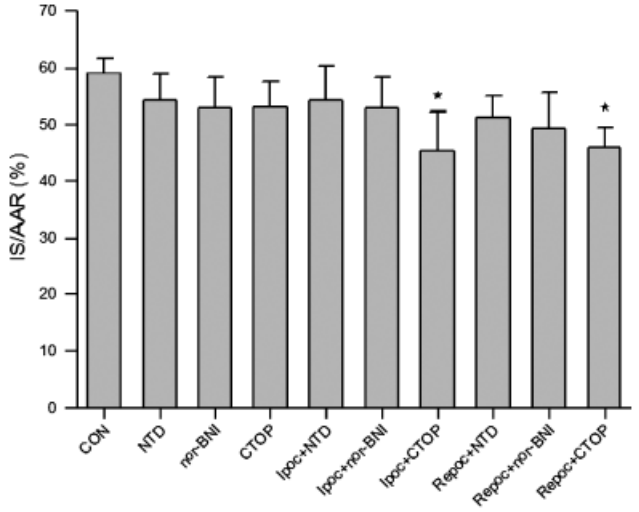

*Fig. 4. Comparison of the infarct size (IS) as a percentage of the area at risk (AAR) for the different treatment groups in the isolated heart preparations. Error bars = ± standard deviations. CON, control group; Ipoc, ischemic post-conditioning; Repoc, remifentanil post-conditioning; NTD, naltrindole; nor BNI, nor-binaltorphimine; CTOP, D-Phe–Cys–Tyr-D-Trp–Orn–Thr–Pen–Thr–NH_2. *P < 0.05 vs. control.*

conditioning may be remotely triggered by inducing ischemia in a distant organ.[30] It is not possible to infer from our results whether remifentanil post-conditioning is an entirely locally and/or remotely triggered as the results are similar both in the intact animal and in the isolated rat.

Most previous observations regarding the relative roles of opioid receptors in post-conditioning have implicated the δ and κ receptors,[4,5,31] as our current data also suggest. Our observations, however, are inconsistent with those from Zatta et al.,[5] where the investigators demonstrated that the effects of ischemic post-conditioning may be inhibited by the μ opioid receptor antagonist CTAP at a dose between 0.09 and 0.19 umol/kg. The dose of 1 mg/kg (0.94 μmol/kg) of CTOP used in this study is higher on a molar basis than the dose of CTAP used by Zatta and colleagues and thus the difference cannot be account for by an insufficient dose. Further inconsistencies are also seen with the δ receptor in post-conditioning. A study evaluating morphine post-conditioning in the isolated heart model demonstrated that its protective effect was not attenuated by the δ receptor antagonist NTD.[32] This finding contrasts with previous work where a specific δ agonist was effective in producing post-conditioning benefits.[19] Therefore, the relative roles of opioid receptors in post-conditioning will require further definition, as it will influence the choice of the exogenous opioid used.

Cardiac post-conditioning has led to exciting prospects for clinical cardiac protection as it removes the Achilles' heel of pre-conditioning, that of timing the intervention before the index ischemic event. Pharmacological post-conditioning can potentially further circumvent the limitations posed by ischemic post-conditioning in the clinical setting. The iatrogenic induction of myocardial ischemia could harm the diseased coronaries or may be arrhythmogenic. Pharmacological post-conditioning may be more versatile as it can easily be applied in the post-cardiopulmonary bypass setting, in patients undergoing thrombolysis as well as coronary angioplasty. Should opioid post-conditioning be shown to be clinically beneficial, remifentanil would indeed be a logical choice for this purpose.

In conclusion, data from this study have confirmed the efficacy of remifentanil post-conditioning as being equal to that of ischemic post-conditioning and both involve the activation of κ and δ receptors. It would be interesting to determine the subcellular mechanisms involved in remifentanil post-conditioning to see whether they are common to those elicited by other opioids, ligands or ischemia. Should obvious differences be apparent, consideration may be made to a multimodal approach to post-conditioning, much analogous to the well-proven practice of multimodal analgesia.

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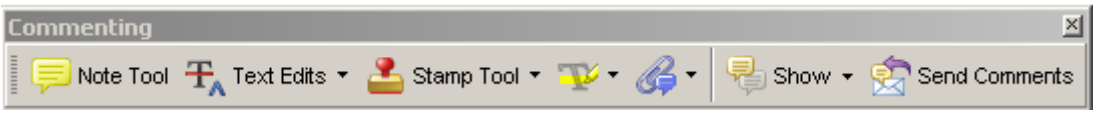

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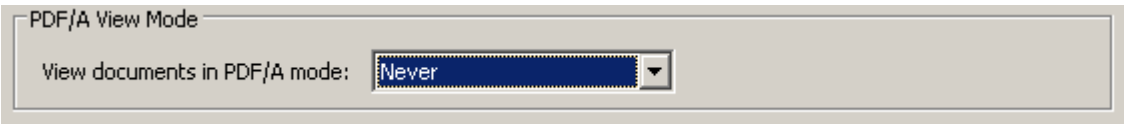

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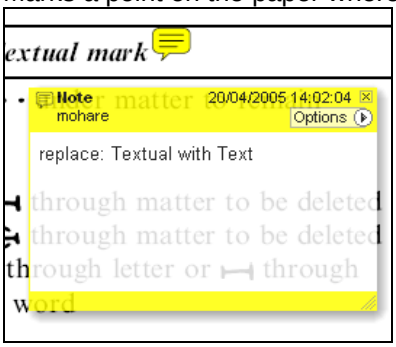

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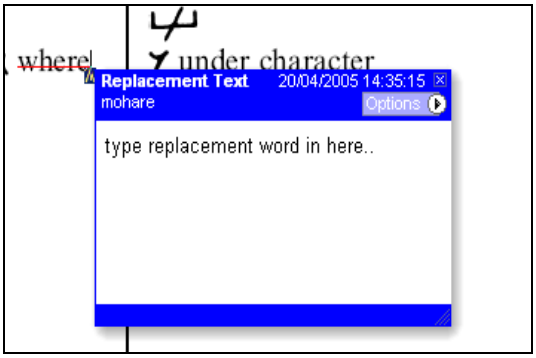

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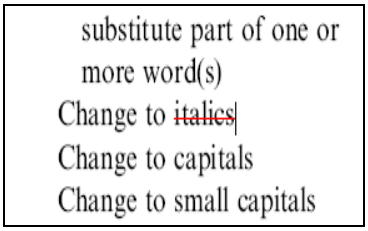

How to use it:

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4. Select Cross Out Text

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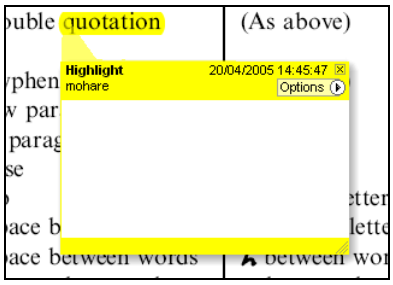

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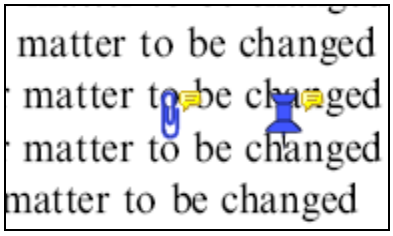

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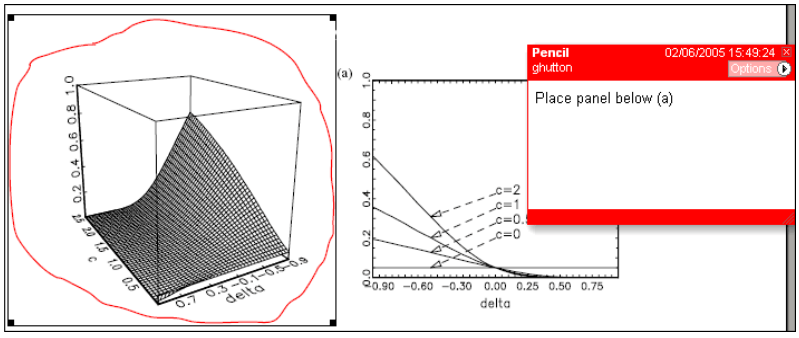

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