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Remote pharmacological post-conditioning by intrathecal morphine: cardiac protection from spinal opioid receptor activation

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Background: Intrathecal morphine pre-conditioning attenuates cardiac ischemia-reperfusion injury via activation of central opioid receptors. We hypothesized that intrathecal morphine also post-conditions the myocardium in the rat.

Methods: Intrathecal morphine at 0.3 $\mu\text{g}/\text{kg}$ (LMPC), 3 $\mu\text{g}/\text{kg}$ (MMPC) or 30 $\mu\text{g}/\text{kg}$ (HMPC) was administered for 5 min before 120-min reperfusion following 30-min ischemia. Infarct size as a percentage of area at risk (IS/AAR) was determined using triphenyltetrazolium staining. MMPC was repeated following the intrathecal administration of nor BNI, NTD, CTOP, or naloxone methiodide (NM), kappa, delta, mu and non-specific opioid receptor antagonists, respectively. The role of peripheral opioid, adenosine and calcitonin gene-related peptide (CGRP) receptors was examined by the intravenous administration of NM, 8- ρ -sulfophenyl theophylline (8-SPT) and human CGRP fragment (CGRP_{8–37}), respectively.

Results: Morphine post-conditioning at all three doses was cardioprotective (IS/AAR of LMPC = $37 \pm 4\%$, MMPC = $35 \pm 5\%$, HMPC = $32 \pm 4\%$, control = $50 \pm 5\%$, $P < 0.01$). The prior administration of opioid receptor antagonists intrathecally, as well as intravenous 8-SPT and CGRP_{8–37} receptor antagonists, abolished this effect (nor BNI+MMPC = $47 \pm 7\%$, NTD+MMPC = $49 \pm 7\%$, CTOP+MMPC = $45 \pm 9\%$, NM+MMPC = $47 \pm 6\%$, 8-SPT+MPC = $46 \pm 5\%$ & CGRP_{8–37}+MPC = $53 \pm 6\%$, $P = 0.63$). However, the intravenous administration of NM did not prevent the protective effect ($34 \pm 4\%$, $P < 0.01$).

Conclusions: Intrathecal morphine administration can induce pharmacological cardiac post-conditioning as it involves opioid receptor centrally but non-opioid receptors peripherally.



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RESTORATION of blood flow following a period of ischemia is known to induce cellular damage in addition to that caused by the ischemia itself.^{1,2} Intervention at the time of reperfusion (post-conditioning) has been clearly demonstrated to ameliorate ischemia-reperfusion (IR) injury and can be achieved by intermittent IR of the area sustaining the ischemic insult, as well as by treatment with a number of drugs.³ Ischemic post-conditioning of another organ or even to the non-ischemic areas within the same organ can also reduce IR injury (remote post-conditioning).⁴ It is difficult, however, to ascertain whether pharmacological post-conditioning is an entirely local or remote phenomenon as both the target and the remote organs are exposed to the conditioning agent.

Cardiac opioid receptors are known to be involved in both ischemic and opioid post-conditioning.^{5–7} Clinically available opioids such as morphine^{7,8} and

remifentanyl^{9,10} have been shown to be cardioprotective when administered intravenously in both a pre-conditioning and a post-conditioning manner. Administration of intrathecal morphine before the index ischemic event can attenuate cardiac IR injury to the same extent as intravenous morphine pre-conditioning.¹¹ However, this protective effect involves the activation of central but not peripheral opioid receptors,¹² pointing to the involvement of non-opioid myocardial receptors in mediating this process. The adenosine receptor was one of the first G-protein-coupled receptors to be linked to the ischemic post-conditioning process¹³ and adenosine is well known to be involved with cardiac protection. The neuropeptide calcitonin gene-related peptide (CGRP) is major neurotransmitter in sensory C fibers¹⁴ and have been shown to be involved in both pre-conditioning and post-conditioning of myocardial tissue.^{15,16} Both of these receptor types are

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widely distributed in cardiovascular tissues. It is possible that some of these receptors may be remotely activated from intrathecal morphine administration. As central opioid receptor agonist activity can remotely pre-condition the myocardium, we hypothesized that intrathecal morphine post-conditioning can also protect the myocardium against IR injury through peripheral non-opioid receptors. We also investigated the possible involvement of the adenosine and CGRP receptors in this process.

Methods

Approval was obtained from our institutional Committee for the Use of Live Animals in Teaching and Research before the commencement of all experimental procedures. A total of 140, 8-week-old Sprague–Dawley rats, weighing 300 ± 25 g, were used for the study. The animals were housed in separate cages, exposed to 12-h light/dark cycles and given free access to food and water.

Intrathecal catheter placement

Following induction of anesthesia using intraperitoneal pentobarbitone (50 mg/kg) and skin preparation with 70% alcohol, a polyethylene-10 catheter (external diameter 0.8 mm and internal diameter 0.4 mm) was inserted through a puncture in the atlanto-occipital membrane. The catheter was then advanced 4 cm to the thoracic spinal cord level according to the method of Yaksh and Rudy,¹⁷ with cerebrospinal fluid (CSF) backflow through the lumen indicating successful placement. After wound closure, the animals were returned to their cages for recovery and observed for the next 3 days. Animals displaying signs of gross motor or sensory deficits were excluded from further study. As an additional confirmation of appropriate catheter placement, Evans blue dye was injected through the intrathecal catheter at necropsy and the spinal cord was inspected for macroscopic evidence of damage.

IR injury

After a minimum of 3 days post-intrathecal catheter placement, an open-chest model was used to produce IR injury. Anesthesia was induced using intraperitoneal pentobarbitone (50 mg/kg) and supplemented (25 mg/kg) if there were signs of light anesthesia indicated by movement of the animal. The lungs were mechanically ventilated

with a Harvard Apparatus Rodent Respirator (Holliston, MA) with room air at 60–70 breaths/min through a tracheostomy tube. Body temperature was monitored and maintained at 37 ± 1 °C using a heating pad. The carotid artery was cannulated with a polyethylene catheter for direct arterial blood pressure monitoring and the right jugular vein was similarly cannulated for drug or saline infusion. Electrocardiogram (lead II) monitoring was achieved using subcutaneous stainless-steel electrodes that were connected via a cable to a PowerLab monitoring system (ML750 PowerLab/4sp with MLT0380 Reusable BP Transducer; AD Instruments, CO Springs, CO).

Once all monitoring and vascular access were established, the heart was exposed via thoracotomy at the left fifth intercostal space, the pericardium was removed and a 6-0 loop of suture was placed with a snare occluder encircling the origin of the left main coronary artery. Regional myocardial ischemia was then induced by tightening the snare and securing the threads with a hemostat, seeking confirmatory signs of cyanosis in the distribution area of the left coronary artery, ST segment changes in the electrocardiogram, and a decrease in mean arterial blood pressure (MAP). Animals developing intractable ventricular fibrillation or severe hypotension (MAP < 30 mmHg) during the ischemic period were excluded from further study without proceeding to post-conditioning treatments. Toward the end of the ischemic period, different post-conditioning maneuvers were performed. The animals underwent a total of 30 min of ischemia followed by 120 min of reperfusion.

Infarct size determination

The hearts were excised and transferred to a Langendorff apparatus at the end of the reperfusion period. The infarct size (IS) was determined as a percentage of the area placed at risk (AAR), using a combination of triphenyltetrazolium and Evan Blue staining, and then quantification was performed using a computerized planimetry technique. The IS/AAR ratio was used to compare the differences between groups.

Study groups and experimental protocol

Two series of experiments were performed concurrently, with the same control group being used for both series. The first series determined the presence of an anti-infarct effect with intrathecal morphine

post-conditioning and compared it with that of ischemic and intravenous morphine post-conditioning. The second series examined the effects that prior administration of opioid, adenosine and CGRP receptor antagonists had on intrathecal morphine post-conditioning. Treatment order followed a predetermined randomized sequence generated for each series. Intrathecal morphine post-conditioning (MPC) was induced by a 5-min infusion of morphine just before reperfusion at doses of 0.3, 3 or 30 $\mu\text{g}/\text{kg}$ (LMPC, MMPC and HMPC, respectively). Positive controls comprised of intravenous morphine and ischemic post-conditioning, while the negative control group received intrathecal normal saline. Intravenous morphine post-conditioning (IVMPC) was achieved using 300 $\mu\text{g}/\text{kg}$ of intravenous morphine and ischemic post-conditioning (IPOC) consisted of three cycles of 30-s reperfusion and 30-s ischemia. In the second series of experiments, 15 nmol of the selective kappa, delta and mu OR antagonists were administered intrathecally 10 min before MPC (3 $\mu\text{g}/\text{kg}$). The compounds were nor-binaltorphimine (nor BNI), naltrindole (NTD) and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂ (CTOP), respectively (Sigma Chemical Company, St Louis, MO). The quaternary non-specific opioid antagonist naloxone methiodide (20 $\mu\text{g}/\text{kg}$) was administered either intrathecally (NM) or intravenously (IVNM). The non-selective adenosine receptor antagonist 8- ρ -sulphonyl theophylline (8-SPT) was administered intravenously at a dose of 7.5 mg/kg¹⁸ and the CGRP receptor antagonist hCGRP₈₋₃₇ was administered at a dose of 0.01 nmol/kg.¹⁹

Each of these compounds was administered, respectively, in the absence of intrathecal morphine to evaluate any infarct-sparing effects. All chemicals were dissolved in normal saline and administered in a volume of 10 μl (Fig. 1).

Statistical analysis

Using this same model of cardiac IR injury, data from previous experiments indicated the expected IS/AAR of the control group to be between 50% and 65% and the magnitude of IS/AAR reduction to be at least 50%. Therefore, five animals per group would be required to yield a power of 80% and a *P* value of 0.05. All data were expressed as mean \pm SD and were obtained from six separate animals per group. For infarct size data, one-way analysis of variance was performed with Student–Newman–Keul tests for multiple comparisons.

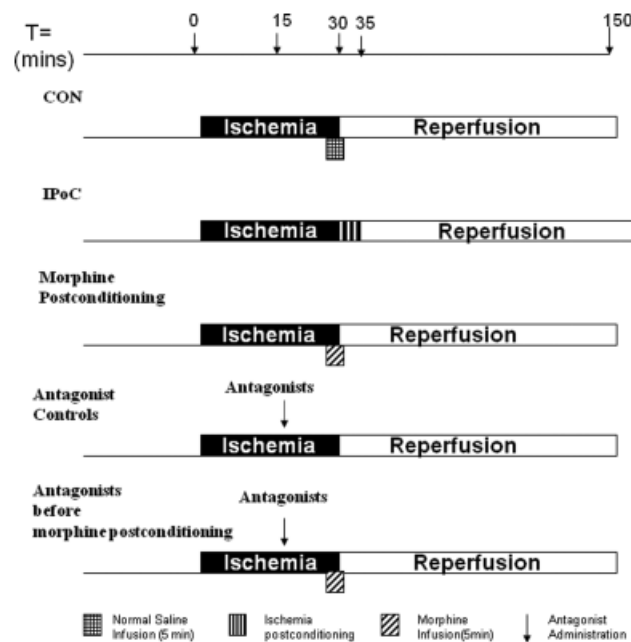


Fig. 1. Bar graph depicting the experimental protocols. Morphine post-conditioning consisted of one of the following regimes: 300 $\mu\text{g}/\text{kg}$ intravenous morphine; 0.3, 3 or 30 $\mu\text{g}/\text{kg}$ intrathecal morphine. Antagonists consisted of one of the following: intrathecal nor BNI, CTOP, NTD or naloxone methiodide; intravenous naloxone methiodide, 8-SPT or CGRP₈₋₃₇. The antagonists were individually administered either alone or before morphine post-conditioning (3 $\mu\text{g}/\text{kg}$). CON, control; IPoC, ischemic post-conditioning.

Hemodynamic data were analyzed using ANOVA for between-group comparisons, with the Bonferroni correction applied for multiple comparisons if significant *F* ratios were obtained. Repeated-measure analysis of variance was used to compare between time points within each group. Statistical analysis was performed using a personal computer software program (SPSS version 16.0 for Windows, Chicago, IL) and a *P* value of <0.05 was considered significant.

Results

A total of 140 animals were used. Six rats suffered neurological damage following intrathecal catheter placement and 14 experienced severe hypotension or ventricular fibrillation and were excluded from further study. These events occurred before administration of any chemicals and therefore not included in the randomized process. The hemodynamic data were comparable at baseline between the groups and are presented in Tables 1 and 2. Ischemic post-conditioning significantly reduced

Table 1

Hemodynamic parameters for the first series of experiments.					
Group	n	Baseline	Ischemia	Treatment	Reperfusion
HR					
Control	6	351 ± 59	351 ± 66	333 ± 67	330 ± 62
LMPC	6	369 ± 61	364 ± 35	327 ± 62†	338 ± 33
MMPC	6	361 ± 39	343 ± 53	307 ± 39†	322 ± 52
HMPC	6	387 ± 26	348 ± 35†	341 ± 52†	286 ± 52†
IVMPC	6	401 ± 31	350 ± 44†	306 ± 45†	324 ± 40†
POC	6	369 ± 69	333 ± 48†	323 ± 60†	260 ± 34†
MAP					
Control	6	105 ± 24	76 ± 22†	77 ± 19†	93 ± 25
LMPC	6	113 ± 15	71 ± 14†	79 ± 15†	89 ± 8†
MMPC	6	101 ± 9	68 ± 14†	74 ± 7†	88 ± 8
HMPC	6	111 ± 10	75 ± 16†	87 ± 18†	82 ± 13†
IVMPC	6	93 ± 23	61 ± 12†	61 ± 14†	77 ± 25†
IPOC	6	107 ± 12	63 ± 12†	55 ± 10*,†	83 ± 16†

Values were obtained at the end of the respective periods.

* $P < 0.05$ compared with control (between group comparison, one-way ANOVA).

† $P < 0.05$ compared with baseline (within group comparison, repeated measure ANOVA).

HR, heart rate (beats per min); MAP, mean arterial pressure (mmHg); IPOC, ischemic post-conditioning; IVMPC, intravenous morphine post-conditioning; LMPC, low dose intrathecal morphine post-conditioning (0.3 µg/kg); MMPC, medium dose intrathecal morphine post-conditioning (3 µg/kg); HMPC, high-dose intrathecal morphine post-conditioning (30 µg/kg).

the mean arterial pressure (MAP) during the treatment period compared with control.

Intrathecal morphine at all three doses reduced the infarct size compared with the control group (LMPC = 37 ± 4%, MMPC = 35 ± 5%, HMPC = 32 ± 4%, control = 50 ± 5%, $P < 0.01$). There was no difference when the MMPC and HMPC were compared with ischemic (30 ± 6%) and intravenous morphine post-conditioning (29 ± 5%) ($P = 0.13$, Fig. 2). The sole administration of any one of the antagonists did not change the infarct size compared with the control group (Fig. 3) ($P = 0.81$). The prior intrathecal administration of any one of the opioid receptor antagonists as well as the intravenous administration of both 8-SPT and CGRP₈₋₃₇ abolished the protective effect of morphine post-conditioning (nor BNI+MPC = 47 ± 7%, CTOP+MPC = 45 ± 9%, NTD+MPC = 49 ± 7%, NM+MPC = 47 ± 6%, 8-SPT+MPC 46 ± 5% and CGRP₈₋₃₇+MPC 53 ± 6%, $P = 0.63$). However, the intravenous administration of NM did not abolish the protective effect (34 ± 4%, $P < 0.01$) (Fig. 4).

Discussion

Our results show that intrathecal morphine can reduce myocardial infarct size at a fraction of the

intravenous dose when administered *after* the start of an index ischemic event and just before reperfusion. Interestingly, this occurs at a much lower dose than what is required to produce this effect intravenously. Agonism of any one of the three opioid receptor subtypes at the spinal cord can activate the cardioprotective process, as blockade of any one of them is sufficient to attenuate this response. In contrast, peripheral opioid receptors are not involved in this process as intravenous naloxone methiodide, a peripherally restricted non-selective opioid antagonist, failed to block the cardioprotective effect. Rather, other receptors such as adenosine and CGRP receptors are involved as their respective antagonists are able to suppress the cardioprotective response. This pattern of cardiac protection is similar to that achieved with intrathecal morphine pre-conditioning.¹¹ Given the slow and limited ability of intrathecal morphine to reach the systemic circulation,²⁰ the short therapeutic window within which systemic opioid post-conditioning is effective²¹ and the lack of peripheral opioid receptor involvement, it is unlikely that morphine itself is the peripheral trigger. Thus, this is a form of remote pharmacological post-conditioning, with the central nervous system and the heart being the remote and the target organs, respectively. As the receptor types for the remote and the target organs differ, this mode of triggering remote post-conditioning of the heart most likely involves secondary signaling mechanisms.

Although innately powerful, the application of ischemic pre-conditioning to clinical practice is limited by the need to intervene before the ischemic event with an invasive protocol. The concept of remote ischemic post-conditioning, first reported by Kerendi et al.,²² can conveniently overcome both these obstacles. Post-conditioning can also be mimicked by a host of pharmacological agents, including drugs commonly used in the perioperative period such as inhalational anesthetics^{23,24} and opioids, as well as adenosine,²⁵ CGRP,¹⁵ bradykinin²⁶ and erythropoietin.²⁷ As both the target and the remote organs may simultaneously be exposed to the agents, the demarcation between remote and local pharmacological post-conditioning is less distinct, though arguably less important. Classical ischemic pre- and post-conditioning have been shown to share some common pathways.²⁸ It is, therefore, not inconceivable that remote pre- and post-conditioning may similarly share certain characteristics. As for remote ischemic pre-conditioning, existing evidence indicates the possible

Table 2

Hemodynamic parameters for the antagonists experiments.

Group	n	Baseline	Ischemia	Treatment	Reperfusion
HR					
Control	6	351 ± 59	351 ± 66	333 ± 67	330 ± 62
Nor-BNI	6	356 ± 64	283 ± 55*	302 ± 74*	297 ± 56*
CTOP	6	396 ± 41	357 ± 34*	330 ± 47*	312 ± 53*
NTD	6	394 ± 67	354 ± 54*	311 ± 74*	319 ± 80*
NM	6	376 ± 41	353 ± 36	360 ± 33	337 ± 40
IVNM	6	407 ± 26	357 ± 20	340 ± 38	330 ± 44*
IV 8-SPT	6	392 ± 13	333 ± 18*	321 ± 18*	325 ± 30*
IV CGRP ₈₋₃₇	6	381 ± 28	325 ± 27*	332 ± 43*	333 ± 35
Nor-BNI+MPC	6	375 ± 63	387 ± 54	365 ± 52	333 ± 40*
CTOP+MPC	6	372 ± 31	317 ± 38*	308 ± 53*	299 ± 43*
NTD+MPC	6	367 ± 61	371 ± 45	375 ± 22	352 ± 43
NM+MPC	6	394 ± 49	396 ± 44	326 ± 40*	324 ± 57*
IVNM+MPC	6	423 ± 16	389 ± 22	354 ± 28*	328 ± 21*
IV 8-SPT+MPC	6	390 ± 33	356 ± 20	348 ± 14	354 ± 26
IV CGRP ₈₋₃₇ +MPC	6	387 ± 28	354 ± 11	340 ± 19	343 ± 28
MAP					
Control	6	105 ± 24	76 ± 22*	77 ± 19*	93 ± 25
Nor-BNI	6	100 ± 23	57 ± 15*	57 ± 14*	77 ± 19*
CTOP	6	107 ± 25	67 ± 14*	67 ± 9*	78 ± 14*
NTD	6	103 ± 27	70 ± 17*	67 ± 15*	81 ± 28*
NM	6	103 ± 9	80 ± 11*	82 ± 9*	83 ± 13*
IVNM	6	111 ± 18	83 ± 11*	82 ± 19*	91 ± 12
IV 8-SPT	6	95 ± 10	88 ± 4	69 ± 5*	79 ± 8
IV CGRP ₈₋₃₇	6	93 ± 16	76 ± 12	64 ± 9	73 ± 17
Nor-BNI+MPC	6	106 ± 21	75 ± 19*	80 ± 21*	86 ± 13*
CTOP+MPC	6	104 ± 18	64 ± 14*	69 ± 18*	71 ± 16*
NTD+MPC	6	102 ± 26	68 ± 11*	73 ± 11*	76 ± 18*
NM+MPC	6	111 ± 26	83 ± 29*	81 ± 22*	95 ± 26
IVNM+MPC	6	108 ± 17	72 ± 17*	76 ± 13*	101 ± 14
IV 8-SPT+MPC	6	110 ± 18	82 ± 14*	80 ± 11*	88 ± 10*
IV CGRP ₈₋₃₇ +MPC	6	109 ± 15	69 ± 10*	82 ± 9*	91 ± 9

Values were obtained at the end of the respective periods.

**P* < 0.05 compared with baseline (within group comparison, repeated measure ANOVA).HR, heart rate (beats per min); MAP, mean arterial pressure (mmHg); MPC, morphine post-conditioning at 3 µg/kg; nor BNI, nor-binaltorphimine; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂; NTD, naltrindole; NM, naloxone methiodide; IVNM, intravenous naloxone methiodide; 8-SPT, 8-ρ-sulfophenyl theophylline; CGRP₈₋₃₇, human fragment 8–37 of calcitonin gene-related peptide.

involvement of neural, humoral and inflammatory mechanisms in the transfer of protection from the remote to the target organ. Although the mechanisms underlying this transfer of protection with remote post-conditioning are less clear, recent studies suggest characteristics similar to that of its pre-conditioning counterpart.²⁹

A popular theory for remote ischemic pre-conditioning involves the release of humoral substances from the remote organ, which is then transmitted in the blood to the target organ. This concept is supported by the observation that plasma from one pre-conditioned animal affords protective properties when transferred to another, independent of neurogenic pathways and even across species.³⁰ This presupposes circulatory access by the putative protective substance to the target tissue at the time of reperfusion. A similar concept has been proposed in

the case of remote post-conditioning where adenosine has been implicated as one of the circulating mediators.²² The strength of post-conditioning protection appears to be dependent on the volume of tissue exposed to ischemia and the duration of the ischemic stimulus applied to the remote organ.³¹ This observation lends itself to the consideration that a threshold needs to be crossed with the remote ischemic post-conditioning stimulus in order for it to confer cardioprotective properties, perhaps with the threshold correlating with the amount of circulating factors generated and released. Intrathecal morphine itself is not known to generate ischemia of the spinal cord that leads to increased production of circulating factors such as adenosine or bradykinin. It has, however, been shown to induce local adenosine release.³² Therefore, the possibility cannot be discounted that intrathecal opioids may trigger the

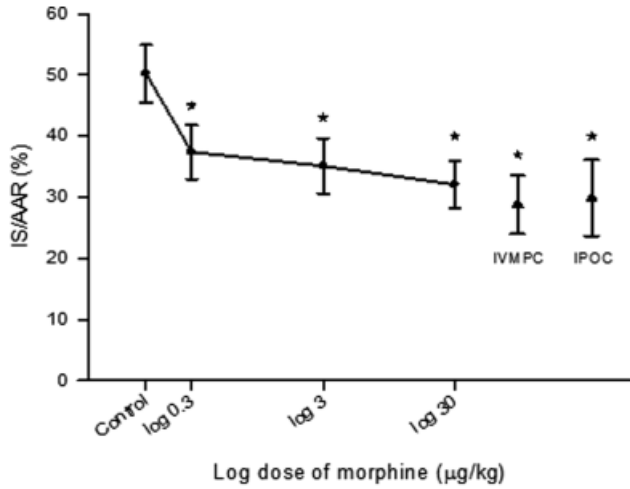


Fig.2. Curve illustrating the relationship between IS/AAR and dose of intrathecal morphine. This is compared with intravenous morphine and ischemic post-conditioning. IS/AAR, infarct size as percentage of area at risk; IV MPC, intravenous post-conditioning; IPOC, ischemic post-conditioning.

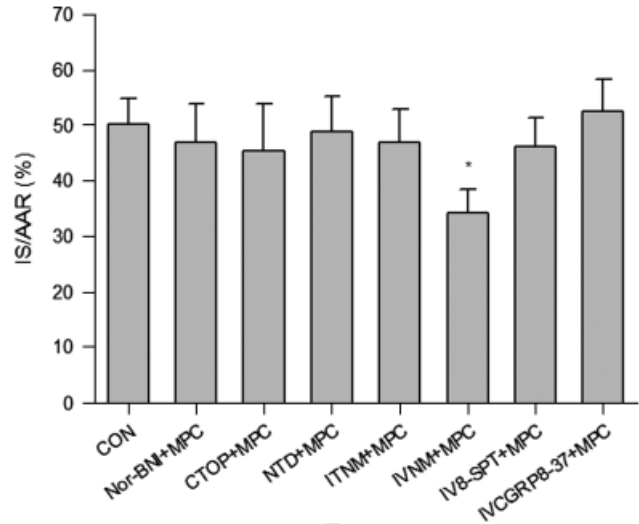


Fig.4. The effect on IS/AAR by the administration of opioid, adenosine and CGRP receptor antagonists before intrathecal morphine post-conditioning (3 µg/kg). Data expressed as percentage ± SD. ANOVA was used for analysis. IS/AAR, infarct size as percentage of area at risk; MPC, morphine post-conditioning at 3 µg/kg; Nor BNI, Nor-binaltorphimine; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂; naltrindole (NTD); NM, naloxone methiodide; IVNM, intravenous naloxone methiodide; 8-SPT, 8-ρ-sulfophenyl theophylline and CGRP₈₋₃₇, human fragment 8-37 of calcitonin gene-related peptide. *P < 0.01 compared with control.

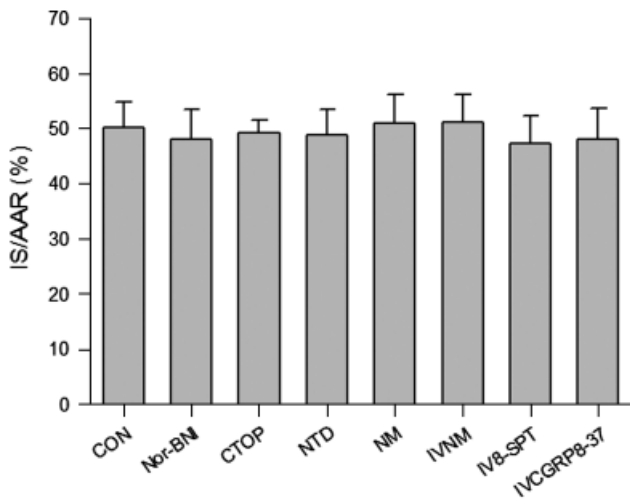


Fig.3. The effect on IS/AAR by opioid, adenosine or CGRP receptor antagonist. Data expressed as percentage ± SD. No difference was detected between any of the group compared with control. ANOVA was used for analysis. IS/AAR, infarct size as percentage of area at risk; MPC, morphine post-conditioning at 3 µg/kg; Nor-BNI, Nor-binaltorphimine; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH₂; naltrindole (NTD); NM, naloxone methiodide; IVNM, intravenous naloxone methiodide; 8-SPT, 8-ρ-sulfophenyl theophylline and CGRP₈₋₃₇, human fragment 8-37 of calcitonin gene-related peptide.

release of such factors from distal organs or nerve endings. From our results, it is possible that peripheral adenosine receptors are involved as the non-specific adenosine receptor antagonist 8-SPT does not cross the blood-brain barrier.³³

Inflammatory activity from neutrophils contribute to the extension of the infarct following

reperfusion.³⁴ Systemic morphine has anti-inflammatory properties³⁵ and, when administered as a pre-conditioning agent in rat models of myocardial infarction, it can attenuate neutrophil-endothelium interactions, a necessary component for tissue injury.³⁶ Although thought to be peripherally mediated, a central mechanism of the anti-inflammatory effect of morphine has been proposed and segmental action of intrathecal morphine has been shown to inhibit inflammatory paw edema.³⁷ However, in that same study, intrathecal morphine had no effect on myeloperoxidase levels, a marker of neutrophil activity, implying a lesser role for neutrophils in its peripheral anti-inflammatory effects. Therefore, if anti-inflammatory effects contribute to infarct sparing with intrathecal morphine post-conditioning, it may not necessarily be by altering neutrophil activity.

As ischemic and pharmacological post-conditioning can also be elicited in isolated heart models,^{13,30,38} neural involvement appears to be not absolutely necessary in remote post-conditioning. However, remote pre-conditioning research has demonstrated that the interruption of autonomic transmission can abrogate the effect of protective ischemia.^{39,40} Similar results have not been

demonstrated for remote post-conditioning. However, CGRP is a neurotransmitter that is released by capsaicin-sensitive sensory nerves and its antagonism abrogates the infarct-sparing effect of intrathecal morphine. This would suggest that an involvement of peripheral CGRP receptors as the antagonist CGRP₈₋₃₇ is not known to freely penetrate the blood-brain barrier.⁴¹ Therefore, the results from this study suggest a contribution of the central nervous system as an initiator of myocardial protection. Whether it may participate in other forms of remote pre- or post-conditioning remains to be determined.

Many aspects of remote post-conditioning remain elusive.^{3,42} Like other complex physiological systems, multiple pathways with inbuilt redundancy may exist with the system of innate protection from IR and the central nervous system may play an integral part either in triggering or in transmitting the protective signals. Multimodal treatment of hypertension and pain are well established in clinical practice and work adopting this approach to cardiac protection has demonstrated augmented response for both pre- and post-conditioning.⁴³⁻⁴⁵ This implies that maximal protection may not be achievable by a single approach. A greater understanding of the triggering mechanisms therefore would potentially enable one to harness the maximal protective effects from myocardial conditioning strategies.

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