British Journal of Anaesthesia **105** (5): 589–95 (2010)
Advance Access publication 6 August 2010 · doi:10.1093/bja/aeq213

BJA

Ischaemic and morphine-induced post-conditioning: impact of mK_{Ca} channels

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Key points

- The heart can be protected from an ischaemic insult by short exposure to ischaemia or morphine before or after the ischaemic insult.
- By using an inhibitor, these authors showed that like preconditioning, post-conditioning occurs via the activation of calcium-sensitive potassium channels in mitochondria.
- An important role for mitochondria in cardioprotection is shown.

Background. Mitochondrial calcium-sensitive potassium (mK_{Ca}) channels are involved in cardiac preconditioning. In the present study, we investigated whether also ischaemic-, morphine-induced post-conditioning, or both is mediated by the activation of mK_{Ca} channels in the rat heart *in vitro*.

Methods. Animals were treated in compliance with institutional and national guidelines. Male Wistar rats were randomly assigned to one of seven groups (each $n=7$). Control animals were not further treated. Post-conditioning was induced either by 3×30 s of ischaemia/reperfusion (I-PostC) or by administration of morphine (M-PostC, 1 μM) for 15 min at the onset of reperfusion. The mK_{Ca}-channel inhibitor paxilline (1 μM) was given with and without post-conditioning interventions (M-PostC+Pax, I-PostC+Pax, and Pax). As a positive control, we determined whether direct activation of mK_{Ca} channels with NS1619 (10 μM) induced cardiac post-conditioning (NS1619). Isolated hearts underwent 35 min ischaemia followed by 120 min reperfusion. At the end of reperfusion, infarct sizes were measured by triphenyltetrazolium chloride staining.

Results. In the control group, infarct size was 53 (5)% of the area at risk. Morphine- and ischaemic post-conditioning reduced infarct size in the same range [M-PostC: 37 (4)%, I-PostC: 35 (5)%; each $P<0.05$ *vs* control]. The mK_{Ca}-channel inhibitor paxilline completely blocked post-conditioning [M-PostC+Pax: 47 (7)%, I-PostC+Pax: 51 (3)%; each $P<0.05$ *vs* M-PostC and I-PostC, respectively]. Paxilline itself had no effect on infarct size (NS *vs* control). NS1619 reduced infarct size to 33 (4)% ($P<0.05$ *vs* control).

Conclusions. Ischaemic- and morphine-induced post-conditioning is mediated by the activation of mK_{Ca} channels.

Keywords: heart; ischaemia; ischaemic preconditioning; morphine; potassium channels; rat

Accepted for publication: 16 June 2010

Pre- and post-conditioning are potent measures to protect the heart against the consequences of ischaemia and reperfusion.[1] Cardioprotection by pre- and post-conditioning can be induced by various stimuli, for example, brief cycles of ischaemia,[2 3] but also pharmacologically, for example, with morphine.[4 5] The mechanisms by which opioids protect the myocardium are common to ischaemic preconditioning. The opening of mitochondrial ATP-sensitive potassium (mK_{ATP}) channels, which are involved in the regulation of mitochondrial functions, is a key step mediating the cardioprotective effects of both ischaemic- and morphine-induced preconditioning. Next to the activation of mK_{ATP} channels,[6] preconditioning seems to be mediated by another class of K^+ channels, the mitochondrial calcium-sensitive potassium (mK_{Ca}) channel.[7 8] In a recent study, Cao and colleagues[9] showed that mK_{Ca} channels are involved in ischaemic preconditioning. Furthermore, these authors demonstrated that the activation of K_{Ca} channels confer cardioprotection independently of mK_{ATP} channels and vice versa.[9] Nothing is known about the involvement of K_{Ca} channels in ischaemic- and morphine-induced post-conditioning.

Here, we hypothesize that ischaemic- and morphine-induced post-conditioning is, as preconditioning, mediated by the activation of K_{Ca} channels.

Methods

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Institutional Committee for Animal Care and Use (Academic Medical Centre Amsterdam, The Netherlands).

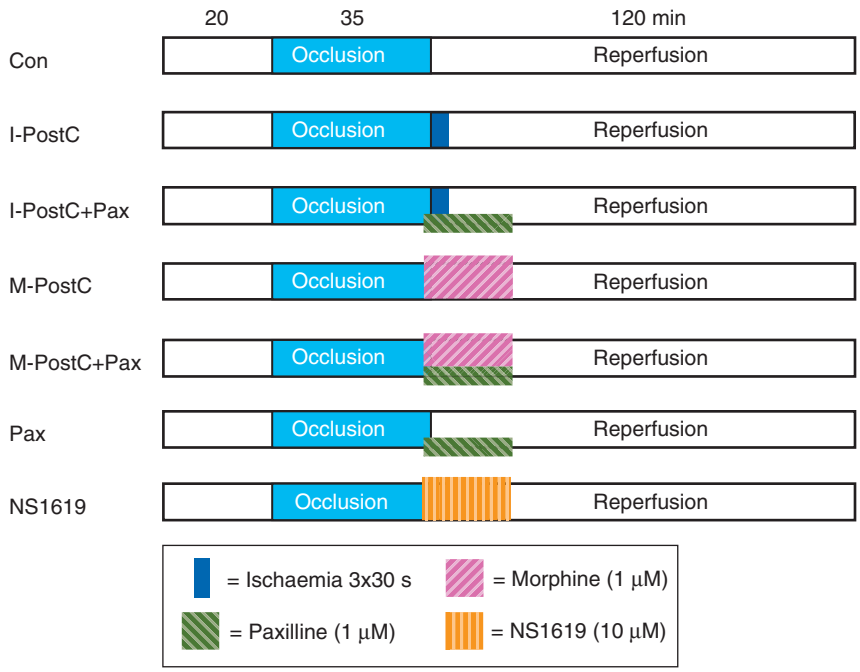

Fig 1 Experimental protocol. Con, control; I-PostC, ischaemic post-conditioning; M-PostC, morphine post-conditioning; Pax, paxilline; NS1619, mK_{Ca}-channel activator.

Chemicals and reagents

Morphine–HCl was purchased from Centrafarm (Etten-Leur, The Netherlands). All other chemicals were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands).

Surgical preparation

Male Wistar rats (Charles River, The Netherlands) were used for these studies. Rats were maintained on a 12:12 light/dark schedule (lights on at 06:00 h) with food and water provided *ad libitum*. The rats were acclimatized to the local animal facility for at least 7 days before the experiment. Rats were anaesthetized with *S*-ketamine (150 mg kg^{-1} i.p.). Before starting surgery, adequate depth of anaesthesia was verified by the absence of reaction after painful stimuli. After thoracotomy, hearts were quickly excised and mounted on a Langendorff system and were perfused at constant pressure (80 mm Hg) with the Krebs–Henseleit solution containing (in mM) 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 0.5 EDTA, 2.25 $CaCl_2$, 11 glucose, 1 lactate, and 0.1 pyruvate at 37 °C. A fluid-filled balloon was inserted into the left ventricle and end-diastolic pressure was set at 1–4 mm Hg. All hearts underwent a stabilization period of 20 min. Heart rate, myocardial function (isovolumetric left ventricular pressure), coronary flow, left ventricular end-diastolic pressure, and dP/dt_{max} were measured continuously. The time of maximal ischaemic contracture and the level of maximal ischaemic contracture were determined in each experiment by checking the course of contracture development during index ischaemia and by selecting the time point (measured from the onset of index ischaemia) when the contracture reached its highest level. Arrhythmic intervals were not used for data analysis. The rate–pressure product was calculated as heart rate × (maximal left ventricular pressure − left ventricular end-diastolic pressure).

Experimental design

Hearts were assigned to one of the seven experimental groups (each $n=7$, Fig. 1). Hearts from all groups underwent 35 min of global ischaemia by stopping coronary perfusion (no-flow ischaemia), followed by 120 min of reperfusion. In the control group, hearts underwent no further intervention. Ischaemic post-conditioning (I-PostC) was induced by three cycles of 30 s ischaemia/reperfusion at the onset of reperfusion.[3 10] To investigate whether morphine induces post-conditioning, 1 μM morphine–HCl[11] (M-PostC) was given over 15 min starting with the reperfusion period. To test whether the cardioprotective effects are mediated by mK_{Ca}-channel activation, we combined both the groups with the mK_{Ca}-channel inhibitor paxilline. The concentration of 1 μM[9 12] was chosen based on the current literature (I-PostC+Pax, M-PostC+Pax). Paxilline was also given without any other treatment (Pax). As a positive control group, we determined whether direct activation of mK_{Ca} channels with NS1619 (10 μM)[9] induces cardiac post-conditioning (NS1619). Morphine was dissolved in NaCl (0.9%) and paxilline and NS1619 were dissolved in DMSO and separately infused into a mixing chamber placed in the perfusion system.