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High dose remifentanil increases myocardial oxidative stress and compromises remifentanil infarct-sparing effects in rats

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ABSTRACT

Chronic administration of high dose opioids such as morphine is known to create intracellular oxidative stress via an opioid receptor dependent mechanism and this can interfere with cellular function. This study aimed at examining whether such changes can occur following short term exposure to high concentration of remifentanil, a potent short acting opioid. We conducted a experimental study using rat myocardium and systematically quantified tissue levels of superoxide anions, malondialdehyde (MDA) and nitrotyrosine following exposure to increasing duration (15 min, 1 or 2 h) or escalating doses of remifentanil (1 μg, 5 μg, 10 μg or 20 μg/kg/min). Concurrently the susceptibility of the heart to ischaemia reperfusion injury was assessed under the similar conditions. For any given duration of remifentanil infusion, there was increasing superoxide anions generated as the dose of remifentanil was increased. MDA concentrations were significantly increased when the animal was exposed to 10 μg/kg/min for 2 h or 20 μg/kg/min for any duration. There was a trend towards an increased nitrotyrosine concentration with increasing dose of remifentanil, becoming significant when the dose was 20 μg/kg/min. The infarct limiting ability of remifentanil was compromised when the dihydroethidium fluorescence positive cell percentage exceeded 50%, MDA concentration greater than 2 nmol/mg of protein and nitrotyrosine content exceeding 1.5 μg/mg of protein. Short term high dose opioid exposure can induce oxidative changes seen previously only with chronic opioid use and this high oxidative stress environment corrupts the heart's sensitivity to be preconditioned by opioids.

1. Introduction

Administration of high doses of opioids such as morphine is known to create intracellular oxidative stress and is associated with cytotoxicity or apoptotic effects in a number of different cell lines, as well as impairing endothelial function (Bhat et al., 2004; Lam et al., 2007; Patel et al., 2003; Zhang et al., 2004b). Morphine induced free radical production seems to involve opioid receptors as it can be blocked by naloxone, a non-specific opioid receptor antagonist (Singhal et al., 1994). The use of high dose morphine is not frequently used in an acute clinical setting due to the likelihood of prolonged respiratory depression in the recipient (McPherson and Yao, 2001). Consequently, high doses morphine is usually only encountered during chronic administration, when significant tolerance has developed to the respiratory depressant effects. However, the advent of potent short acting opioids such as remifentanil facilitates very high doses of opioids to be given within a short period of time, such as during surgery, while allowing rapid dissipation of effects. This has led to the observation acute opioid tolerance that was previous only seen with chronic use of opioid. Whether such acute exposure to high dose short-acting opioids induces a detrimental change in cellular oxidative state is not known. Oxidative free radicals in large amounts are known mediators of damage in ischaemia reperfusion injury in the heart and other organs. In contrast, small amounts of free radicals are known to participate in the signalling of innate protective processes such as pre and post-conditioning, a process during which cells are rendered more resistant to ischaemia reperfusion injury (Tritto et al., 1997; Vanden Hoek et al., 1998). Opioids such as morphine and remifentanil confer cardiac and other organ protection in animal models (Schultz et al., 1996; Yang et al., 2011; Zhang et al., 2004a). We hypothesised that short term exposure to high doses of remifentanil can increase cellular oxidative stress similar to that seen with chronic opioid use and this high oxidative stress environment corrupts the heart's sensitivity to be preconditioned by opioids.

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2. Materials and methods

Ethical approval for this study was provided by the local Committee on the Use of Live Animal in Teaching and Research. Male Sprague-Dawley rats weighing 250–300 g were used in these experiments and were housed in separate cages and they were given free access to food and water except before study and exposed to 12 h light and dark cycles. Separate groups of animals were exposed to continuous infusions of remifentanil at escalating doses for different duration, or to remifentanil or to ischaemic preconditioning. The hearts from four animals were harvested at the end of this exposure and assessed for the degree of oxidative and nitrostrative stress, the levels of which was compared with sham animals, *without* undergoing ischaemia reperfusion injury. Six animals from each treatment group then proceeded to undergo cardiac ischaemia and reperfusion injury. The hearts were harvested to assess for infarct size relative to at risk zone. The degree of myocardial damage was compared to control animals which received only saline infusion prior to the prolonged ischaemia. In a second series of experiments, hearts exposed to 2 h infusion of remifentanil at escalating doses from four animals were assessed for their superoxide dismutase activity as well as the 8-Hydroxy-2'-deoxyguanosine (8-OHdG) levels.

2.1. Surgical preparation

An open chest rat model was used and the methods have been described in detail previously (Li et al., 2009). In brief the rats were anesthetised by intraperitoneal administration of pentobarbitone (50 mg/kg body weight) without use of muscle relaxants and maintained by repeat doses of 25 mg/kg of pentobarbitone every 60–90 min as necessary. Once anaesthetised, tracheotomy and tracheal intubation were performed and mechanical ventilation was established using a Harvard Apparatus Rodent Respirator (Harvard Apparatus, Boston, MA). A left thoracotomy was performed to expose the heart and a 6-0 Prolene loop, along with a snare occluder, was placed at the origin of the left coronary artery. After surgical preparation, the rat was allowed to stabilise for 15 min. Thirty min of regional ischaemia was induced by pulling the snare and securing the threads with a mosquito haemostat. Rats were omitted from further data analysis if severe hypotension (arterial mean blood pressure less than 30 mmHg) or intractable ventricular fibrillation occurred. Haemodynamic variables were continuously monitored via direct cannulation of the carotid artery and electrocardiogram was monitored 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).

2.2. Study groups and experiments protocols

As shown in Fig. 1, the rats were randomly assigned to one of the following groups:

1. Sham group (SHAM): only had heart exposure and sutures applied without ischaemia reperfusion.
2. Control group (CON): intravenous saline given before induction of myocardial infarction.
3. Ischaemic preconditioning group (IPC): three cycles of 5 min ischaemia with 5 min reperfusion prior to myocardial infarction.
4. Remifentanil preconditioning group (RPC): three cycles of 5 min infusion of remifentanil at 5 μg/kg/min interspersed with 5 min infusion free periods; and
5. Remifentanil exposure groups: remifentanil infusion administered at doses of 1 μg (R_1), 5 μg (R_5), 10 μg (R_{10}) or 20 μg/kg/min (R_{20}) respectively, for 15 mins (T_{15}), 1 h (T_{60}) or 2 h (T_{120}) at each respective dose. Remifentanil was dissolved in normal saline and infused by a micro pump at a speed of 1.8 ml/h. All infusions were completed before the induction of myocardial infarction or the removal of the heart for tissue sampling.

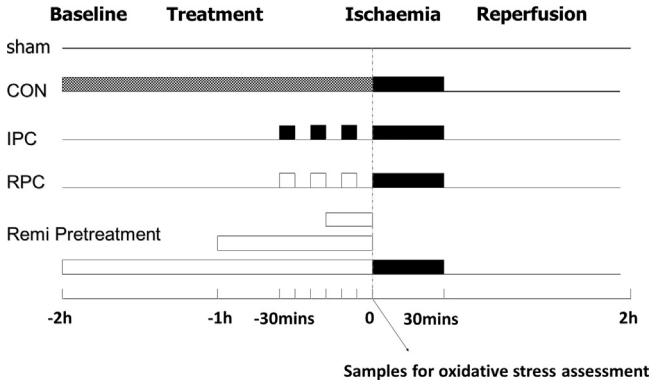

Fig. 1. Time lines depicting the experimental protocols. Hearts from 4 animals were used from each group to assess for oxidative stress and a further 6 animals underwent ischaemia reperfusion injury. Solid boxes represent periods of ischaemia and stripped boxes represent periods of remifentanil infusion. CON=control; IPC=ischaemic preconditioning; RPC=remifentanil preconditioning.

2.3. Tissue superoxide anion by dihydroethidium fluorescence

Superoxide anion generation from the myocardium was determined using dihydroethidium (DHE) fluorescence. DHE is a cell permeable stain that is rapidly oxidised to fluorescent ethidium by superoxide anions (Peshavariya et al., 2007), which is then intercalated into DNA and is, therefore, a presumptive marker of intracellular superoxide anion generation. It was injected into the animal prior to the drug infusion. Immediately after the remifentanil infusion, hearts ($n=4$) were removed and a sample from the area supplied by the left coronary artery was excised and embedded for cryosectioning. These hearts were not subjected to triphenyltetrazolium staining. About 20 μm tissue sections were cut using a Hacker-Bright cryostat, thaw-mounted on Fisher-Plus slides (Fisher Scientific), and stained with 10 μM DHE at 37 °C for 30 min. The fluorescent image was obtained using a fluorescence microscope with a 585-nm long-pass filter attached to an image analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD). Superoxide generation in the myocardium was expressed as red fluorescence. Four determinations were performed in each group. DHE staining was quantified by counting the number of positive cardiomyocyte nuclei in 10 random fields (magnification 400 ×). The results are expressed as the number of positive nuclei as a percentage of the total nuclei in the randomly selected field (Fiordaliso et al., 2006).

2.4. Determination of myocardium malondialdehyde level

Malondialdehyde (MDA), a presumptive marker of oxidant-mediated lipid peroxidation (Di Pierro et al., 1992), was quantified to estimate the extent of lipid peroxidation in the at risk myocardium as described (Liu et al., 2008). Immediately following preconditioning or remifentanil exposure, the hearts were removed and myocardial tissue of a similar area supplied by the left coronary artery was excised and frozen in liquid nitrogen. After the tissue was homogenated, the total concentration of protein was determined by a spectrophotometric absorbance method and the MDA was measured using a colorimetric method from a commercially available assay kit (Nanjing Jiancheng technology, China). The results are expressed as nmol of MDA per mg of protein.