Life Sciences 93 (2013) 373–379

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Role of endogenous opioid peptides in the infarct size-limiting effect of adaptation to chronic continuous hypoxia

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ARTICLE INFO

Article history:
Received 26 March 2013
Accepted 15 July 2013

Keywords:
Chronic hypoxia
Myocardial infarction
Cardioprotection
Opioid peptides
Opioid receptors

ABSTRACT

Aims: The objective of this study was to examine the involvement of endogenous opioid peptides and opioid receptor (OR) subtypes in the cardioprotective effect of adaptation to chronic hypoxia in rats.
Main methods: Rats were exposed to continuous normobaric hypoxia (CNH; 12% oxygen) for 3 weeks. Myocardial ischemia was induced by 20-min coronary artery occlusion followed by 3-h reperfusion in anesthetized open-chest animals. Various OR antagonists were administered to rats prior to ischemia. The size of myocardial infarction and the incidence of ischemic ventricular arrhythmias were assessed. Myocardial and plasma concentrations of opioid peptides (met-enkephalin, β-endorphin, and endomorphins) were determined.
Key findings: Adaptation to CNH significantly increased myocardial and plasma concentrations of opioids, potentiated their further elevation by ischemia/reperfusion, and reduced myocardial infarct size, but it did not affect the incidence of ischemic arrhythmias. The infarct size-limiting effect of CNH was abolished by OR antagonists naltrexone (non-selective), naloxone methiodide (non-selective peripherally acting), TIPP[ψ] (δ-OR), naltriben (δ_2-OR), or CTAP (μ-OR), while BNTX (δ_1-OR) and nor-binaltorphimine (κ-OR) had no effect.
Significance: The results suggest that the infarct size-limiting effect afforded by adaptation to CNH is mediated by activation of peripheral δ_2- and μ-ORs by elevated levels of endogenous opioid peptides.

Introduction

Pharmacological activation of opioid receptors (ORs) has been convincingly shown to ameliorate manifestations of myocardial injury caused by acute ischemia/reperfusion (I/R) insult. It is also generally accepted that endogenous opioid peptides are important players in temporal cardioprotection induced by various modes of conditioning. However, the involvement of OR subtypes in improved ischemic tolerance with respect to their localization is still a rather controversial issue. Most of studies suggest that the activation of peripheral δ- or κ-OR by endogenous or exogenous opioids is associated with ischemia-tolerant phenotype (reviewed by Gross, 2003; Peart et al., 2005). While centrally located μ-OR can also mediate some forms of cardioprotection (Gross et al., 2012; Wong et al., 2012), there is little consensus for the role of peripheral μ-OR (Zatta et al., 2008).

According to a recent report, mice treated with morphine for 5 days exhibited marked cardioprotection, which persisted longer than any mode of conditioning. This clinically attractive form of protection, termed sustained ligand-activated preconditioning, was mediated specifically by δ-OR (Peart et al., 2011). It is well known that long-lasting ischemic tolerance can be also achieved by adaptation to various stressful conditions such as chronic hypoxia. Rats adapted to intermittent hypoxia exhibited significant infarct-sparing effect persisting as long as 5 weeks after the cessation of hypoxic exposure (Neckar et al., 2004). Although this form of myocardial salvation appears to share some protective components with preconditioning, its detailed mechanism remains poorly understood (reviewed by Ostadal and Kolar, 2007). The only report dealing with opioids demonstrated that pretreatment with the selective δ-OR antagonist, TIPP[ψ], abolished the suppression of ischemic and reperfusion ventricular arrhythmias afforded by adaptation to intermittent hypoxia (Lishmanov et al., 2003). It is unknown, whether endogenous opioid system plays a role in chronic hypoxia-induced myocardial protection against irreversible I/R injury.

The purpose of this study was, therefore, to examine the role of OR subtypes in the infarct size-limiting effect of chronic hypoxia by using both non-selective and selective OR antagonists. In addition, myocardial and plasma concentrations of major opioid peptides were determined

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http://dx.doi.org/10.1016/j.lfs.2013.07.018

in animals subjected to I/R or shame operation. Experiments were performed on rats adapted to continuous hypoxia that exhibit improved tolerance to myocardial infarction but not to ventricular arrhythmias associated with I/R (Neckar et al., 2003, in press).

Materials and methods

Animals

The experimental protocol was approved by the Ethical Committee of the Research Institute of Cardiology, Siberian Branch of the Russian Academy of Medical Sciences, and it conformed to the EU Direction 2010/63/EU. Male Wistar rats weighing 250–300 g were housed at 23 ± 1 °C with a relative humidity of 60–70% and a light/dark cycle of 12 h with free access to water and standard rat chow. Rats were exposed to continuous normobaric hypoxia (CNH) for 3 weeks in a chamber (1.5 m^3) equipped with a hypoxia generator Bio-Nova-204G4R1 (NTO Bio-Nova, Moscow, Russia). Concentrations of oxygen and carbon dioxide in the chamber were continuously measured by TCOD-IR and OLC 20 sensors, and maintained at 11.75–12.25% and 0.03%, respectively, by a controller MX32 (Oldham, France). Normoxic rats were used as a control group.

Myocardial ischemia/reperfusion

The rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.; Sanofi-Aventis, France). A tracheotomy was performed, and the lungs were ventilated (modified model RO-6, Krasnogvardeets, St. Petersburg, Russia) with room air. Atelectasis was prevented by maintaining a positive end-expiratory pressure of 5–10 mm H_2O. Arterial pH, PCO_2, and PO_2 were monitored throughout the experiment by a blood gas analyzer (Stat Profile M, Nova Biomedical, Waltham, MA, USA) and maintained within a normal physiological range by adjusting the respiratory rate and/or tidal volume. Body temperature was maintained at 37 °C by a heating pad. Blood samples were taken from the tail. Hematocrit was determined by a capillary micromethod and hemoglobin content was measured by a hemichrome method using a commercial kit (Vector Best, Novosibirsk, Russia). A number of erythrocytes were calculated as well (Menshikov et al., 1987).

The left femoral artery was cannulated for blood pressure, heart rate, and blood gases measurements. Blood pressure and standard peripheral lead electrocardiogram (ECG) recordings were performed with a MP35 apparatus and a computer using the BSL PRO 3.7.3 software (Biopac Systems Inc., Goleta, CA, USA). The right femoral vein was cannulated for the administration of pharmacological agents or vehicles.

Regional myocardial I/R was induced as described by Neckar et al. (2002). Left thoracotomy was performed and after 10-min stabilization, regional myocardial ischemia was induced by tightening a ligature (6-0 Prolene) placed around the left anterior descending coronary artery near its origin. Characteristic changes in the configuration of the ECG and a transient decrease in blood pressure verified the coronary artery occlusion. After a 20-min occlusion period, the ligature was released and reperfusion of previously ischemic tissue continued.

Infarct size determination

At the end of 3-h reperfusion, the hearts were excised and perfused with saline through the cannulated aorta. The area at risk and the infarct size were delineated by staining with 5% potassium permanganate and 1% 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich, MO, USA), respectively (Neckar et al., 2002). The right ventricle was separated and the LV was cut perpendicularly to its long axis into slices 1 mm thick and stored overnight in 10% neutral formaldehyde solution. The infarct size (IS), the size of the area at risk (AAR) and the size of the LV were determined by a planimetric method using Scanjet G4050 scanner (Hewlett-Packard, Palo Alto, USA). The IS was normalized to the AAR (IS/AAR) and the size of the AAR was normalized to the left ventricle (AAR/LV).

Incidence of arrhythmias

Ventricular arrhythmias were recorded from the ECG signal during 20-min coronary artery occlusion. The incidences of single premature ventricular complexes including salvos, ventricular tachycardia and ventricular fibrillation were evaluated separately.

Opioid receptor antagonists

Three series of experiments were performed using normoxic and chronically hypoxic rats pretreated with various OR antagonists and appropriate vehicle-treated controls. The non-selective OR antagonist, naltrexone hydrochloride, and the non-selective peripherally acting OR antagonist, naloxone methiodide, were administered at a dose of 5 mg/kg (Maslov et al., 2009; Schultz et al., 1997). The selective δ-OR antagonist, TIPP[ψ] (H-Tyr-Ticψ [CH_2NH]Phe-Phe-OH), was given at a dose of 0.5 mg/kg (Lishmanov et al., 2003). The selective δ_1-OR antagonist, BNTX (7-benzylidenenaltrexone maleate), was given at a dose of 0.7 mg/kg (Maslov et al., 2009; Sofuoglu et al., 1993), and the selective δ_2-OR antagonist, naltriben mesylate, was used at a dose of 0.3 mg/kg (Maslov et al., 2009; Kamei et al., 1995). The selective κ-OR antagonist, nor-binaltorphimine (nor-BNI), was administered at a dose 9 mg/kg (Birch et al., 1987; Maslov et al., 2005a, 2005b). The selective μ-OR inhibitor, CTAP (NH_2-D-Phe-c[Cys-Tyr-D-Trp-Arg-Thr-L-Pen]-Thr-NH_2), was used at a dose of 0.1 mg/kg based on our preliminary experiments. All agents were administered intravenously 25 min before coronary artery occlusion, except for nor-BNI, which was given 90 min before occlusion according to Birch et al. (1987).

Naltrexone, naloxone methiodide, CTAP, TIPP[ψ] and nor-BNI were dissolved in saline. Naltriben and BNTX were dissolved in 0.1 ml DMSO and then diluted in 0.9 ml 20% hydroxypropyl-β-cyclodextrin. All solutions were prepared just before use and administered in a volume of 1 ml/kg. Our pilot experiments proved that 20% hydroxypropyl-β-cyclodextrin had no effect on hemodynamics, incidence of ventricular arrhythmias and myocardial infarct size.

CTAP and TIPP[ψ] were synthesized by PolyPeptide Laboratories (San Diego, CA, USA). Naltrexone, naloxone methiodide, naltriben, BNTX, nor-BNI, triphenyltetrazolium chloride and hydroxypropyl-β-cyclodextrin were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Determination of opioid peptides

Rats were subjected to myocardial I/R as described above. Sham-operated animals served as controls. After decapitation, blood was collected into a tube containing EDTA sodium salt (10 mg/ml of blood) and centrifuged at 1600 ×*g* for 15 min at 4 °C. Plasma was collected and frozen at −70 °C for later use. The heart was excised and dissected into the left and right ventricles. Myocardial samples (about 80–100 mg) were obtained from the central part of the left ventricular free wall area supplied by the occluded artery (area at risk), frozen in liquid nitrogen and stored at −70 °C for later use.

Myocardial and plasma concentrations of opioid peptides met-enkephalin, β-endorphin, endomorphin-1 and endomorphin-2 were determined using enzyme immunoassay kits S-1170.0001 β-Endorphin (rat), S-1139.0001 Endomorphin-1, S-1419.0001 Met-Enkephalin, and S-1246.0001 Endomorphin-2 (Peninsula Laboratories, San Carlos, CA, USA). The extraction of opioid peptides was performed according to the manufacturer's instruction using a C18 Sep-column. Briefly, the plasma sample was acidified with an equal volume of 1% trifluoroacetic acid (TFA, solution A) and centrifuged at 6000 ×*g* for 15 min at 4 °C. The frozen myocardium was grinded in a mortar, the obtained powder was