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Role of dynorphin in hypoxic pulmonary hypertension

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ABSTRACT

Previously study showed κ-opioid receptor stimulation with exogenous κ-opioid receptor agonist elicited a protective effect against hypoxic pulmonary hypertension (HPH). However, the effect of endogenous κ-opioid receptor agonist dynorphin A on HPH remains unclear. This study was to determine the role of dynorphin in HPH. Hypoxia for 2 weeks induced HPH. Compared with the HPH group, the HPH + nor-BNI (a selective κ-opioid receptor antagonist) group showed a significant increase in mean pulmonary arterial pressure (mPAP). Exogenous treatment with dynorphin A 1–13 significantly decreased mPAP in HPH rat. In addition, we evaluated the effect of exogenous κ-opioid receptor agonist U50,488H on mPAP. The anti-HPH effect of dynorphin A was less than that of U50,488H. Meanwhile, level of dynorphin A in serum and lung was increased during hypoxia for 2 weeks, while it decreased after hypoxia for 4 weeks. In addition, both the level of ET-1 and AngII were increased during hypoxia. Dynorphin A 1–13 and U50,488H time-dependently relaxed pulmonary artery from both normal and HPH rats. The relaxation of dynorphin A was less than that of U50,488H. Dynorphin A 1–13 inhibited the proliferation of pulmonary artery smooth muscle cells (PASMCs) during hypoxia, which was blocked by nor-BNI. κ-opioid receptor expression increased in PASMCs in both normoxia exposed to dynorphin A 1–13 and during hypoxia. Hypoxia-induced increase was enhanced by dynorphin A 1–13 and abolished by nor-BNI. In conclusion, endogenous dynorphin A released in the early stage of hypoxia plays a protective effect against HPH via stimulation of κ-opioid receptor.

1. Introduction

Pulmonary hypertension is an insidious and complex disease involving both the pulmonary and cardiovascular systems. The signs and symptoms of pulmonary hypertension are nonspecific and subtle. Left untreated patients will experience progressive symptoms, such as dyspnea and right heart failure, culminating in markedly curtailed survival (Gaine, 2000; Oparil et al., 2003; Sandoval, 2006). Various respiratory diseases, such as long-term living in plateau environment and chronic obstructive pulmonary disease (COPD), can lead to hypoxic pulmonary hypertension (HPH) (Heath et al., 1990; Wilkinson et al., 1988; Sutendra and Michela, 2013). HPH is an important pathophysiological stage. However, the pathogenesis of HPH remains unclear, and the therapeutic effect is still unsatisfactory. So, there is an urgent need for developing an efficacious strategy in the treatment of HPH.

HPH is a progressive disorder characterized by abnormally high blood pressure in the pulmonary artery caused by hypoxemia after various respiratory system diseases. Both COPD and living in the plateau for a long time can lead to HPH. Hypoxia-induced pulmonary vasoconstriction and pulmonary vascular remodeling are two key pathophysiologic processes in HPH (Chan and Loscalzo, 2008; Dahal et al., 2010). Long-term hypoxia results in irreversible vasoconstriction in the pulmonary artery and further leads to HPH. All hypoxic pathways ultimately lead to smooth muscle (SM) dysfunction through cellular remodeling (MacLean, 1999; Tuder et al., 2001), and chronic hypoxic stress upsets the balance between the vasoconstrictor (ET-1, AngII) and vasodilator (NO, PGI), triggering endothelial dysfunction and gradually leading to vascular remodeling characterized by proliferation of pulmonary artery smooth muscle cells (PASMCs). Eventually, HPH and right ventricular hypertrophy come into being. Therefore, dilating pulmonary artery, inhibiting smooth muscle cell proliferation are potential strategies for the prevention and treatment of HPH.

It has been demonstrated that endogenous opioid peptide and its receptor play a critical role in the cardiovascular system, of which the main subtype is κ-opioid receptor (Tai et al., 1991). Endogenous opioid peptides can be synthesized and released by the heart, and it modulates cardiac activities directly (Younes et

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al.,2000; Wong and Shan, 2001; Guo et al., 2011). In our previous study, κ-opioid receptor has been identified in pulmonary arteries and κ-opioid receptor expression also increases during hypoxia (Peng et al., 2009). We hypothesize that endogenous dynorphin increases and up regulates κ-opioid receptor during hypoxia. However, the role of dynorphin A in the pathophysiology of HPH has not been reported yet.

Hypoxia-induced pulmonary vasoconstriction and pulmonary vascular proliferation are two key processes in HPH. Our previous studies have demonstrated that U50,488H, a selective exogenous κ-opioid receptor agonist, elicits a definite dilating effect on pulmonary artery and a definite inhibitory effect on PASMCs proliferation (Sun et al., 2006; Zhang et al., 2013). However, whether endogenous κ-opioid peptide, dynorphin A, confers those effects and whether those are mediated by κ-opioid receptor activation remains obscure.

Therefore, the present study is supplementally designed to investigate the role of dynorphin A 1–13 in HPH and its underlying mechanisms.

2. Materials and methods

2.1. Reagents

U50,488H (trans-3,4-dichloro-N-methyl-[2-(1-pyrrolidinyl) cyclohexyl] benzeacetamide), a selective κ-opioid receptor agonist and nor-binaltorphimine (nor-BNI), a selective κ-opioid receptor antagonist were purchased from Tocris Cookson. Dynorphin A 1–13 was purchased from Sigma. All compounds were dissolved in normal saline (0.85% NaCl solution) before use. Rabbit polyclonal immunoglobulin (Ig) G [κ-opioid receptor-1 (H-70)] (sc-9112) (κ-opioid receptor-1 is a rabbit polyclonal antibody raised against amino acids 1–70 of κ-opioid receptor-1 of human origin, and its molecular weight is 58 kDa) was purchased from Santa Cruz Biotechnology, and β-actin primary antibody (sc-1616r) were purchased from Santa Cruz Biotechnology. The secondary antibody (goat anti rabbit antibody) conjugated with HRP was purchased from Boster Company. The Protein Quantitation Kit and chemiluminescence kits were purchased from Pierce Company. Dynorphin A, ET-1 and Ang II ELISA kits were purchased from Uscn life Science & Technology Company.

2.2. Animals

Thirty-six male Sprague-Dawley rats (200 ± 10 g) were purchased from the animal center of the Fourth Military Medical University. Rats were kept under pathogen-free conditions at about 22 °C on a 12-h light–dark cycle with free access to food and water. This study was performed according to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996) and was approved by the Ethics Committee of the Fourth Military Medical University.

2.3. Animal groups and rats HPH model

Sprague Dawley rats were randomly divided into 6 groups: (1) Control group, normoxic group; (2) hypoxia for 1W group, rats were exposed to hypobaric and hypoxic condition for 1 week; (3) hypoxia for 2W group, rats were exposed to hypobaric and hypoxic condition for 2 weeks; (4) hypoxia for 2W+NS group, same as hypoxia for 2W group and NS (normal saline), at 2.0 ml/kg was intraperitoneally injected 10 min prior to hypoxia every other day; (5) hypoxia for 2W+nor-BNI group, same as hypoxia for 2W group and nor-BNI, a selective κ-opioid receptor antagonist, at 2.0 mg/kg was intraperitoneally injected 10 min prior to hypoxia every other day; (6) hypoxia for 4W group, rats were exposed to hypobaric and hypoxic condition for 4 weeks.

The animal model of hypobaric and hypoxic pulmonary hypertension was performed with automatic hypoxia equipment (Li et al., 2009; Wu et al., 2013). Hypoxia was performed for 8 h every day by exposing rats to low pressure and low oxygen (air pressure 50 kPa, oxygen concentration 10%). The control group of rats was placed in room air.

To investigate the effect of dynorphin A 1–13 and U50,488H, animals were injected with dynorphin A 1–13 and U50,48H, at 1.25 mg/kg (Khawaja et al., 1990) through vena femoralis after hypoxia for 2W and hypoxia for 4W.

2.4. Culture of pulmonary artery smooth cells

Rats were anesthetized via intraperitoneal injection with pentobarbital sodium (100 mg/kg, i.p.). The pulmonary arteries of each rat were segregated in a sterile manner. Obtained PASMCs were cultured according to previously reported methods (Peng et al., 2009). Briefly, the outer sphere was peeled and endothecia were shaved slightly to remove endothelial cells. The tunica media was cutted into scraps (1 mm^3) in Dulbecco modified Eagle's medium (DMEM). PASMCs were cultured in DMEM (containing 20% fetal bovine serum, 5% CO_2 at 37 °C). Immunohistochemistry staining of actin in the PASMCs showed that α-actin distributed in cytoplasm. Experiments were performed with 3–6 generation cells.

2.5. Measurement of hemodynamics

After hypoxia, rats were anesthetized via peritoneal injection with pentobarbital sodium (60 mg/kg, i.p.). Supplemental doses of sodium pentobarbital were given when needed to maintain a uniform level of anesthesia. According to Michelakis et al. (2002), Pei et al. (2006) and Li et al. (2013), a micro-catheter was inserted into right ventricle and pulmonary artery through right external jugular vein, and the mean pulmonary arterial pressure (mPAP) was measured.

2.6. Measurement of anti-pulmonary artery hypertension in HPH rats

To determine the effect of dynorphin A 1–13 and U50,488H on HPH, we evaluated the effect of dynorphin A 1–13 and U50,488H on mPAP in HPH rats. dynorphin A 1–13 at 1.25 mg/kg was administered to HPH rats by intravenous injection after hypoxia for 2W and hypoxia for 4W. U50,488H at 1.25 mg/kg was given to HPH rats by intravenous injection after hypoxia for 4W.

2.7. Measurement of serum and lung dynorphin A, ET-1 and AngII levels

Blood and tissues were collected after hypoxia, the blood was centrifuged at 3000*g* for 10 min, and then the serum was collected. The lung were minced and homogenized in buffer (1 M PBS which contains 1% protease inhibitor cocktail) by a Heidolph DIA900 tissue homogenizer (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) (Li et al., 2007). The homogenate was centrifuged with 12,000*g* for 20 min at 4 °C. The supernatant was decanted, and total protein level was obtained with a BSA assay kit. After the above protocol, the level of dynorphin A, ET-1 and AngII were determined with dynorphin A, ET-1 and AngII ELISA kit, respectively. Determination was carried on strictly according to instruction of production.