Peptides 31 (2010) 1772–1778

Contents lists available at ScienceDirect

Peptides

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

Hemodynamic effect of apelin in a canine model of acute pulmonary thromboembolism

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ARTICLE INFO

Article history:
Received 30 March 2010
Received in revised form 7 June 2010
Accepted 7 June 2010
Available online 16 June 2010

Keywords:
Apelin
Acute pulmonary embolism
Lung
Pulmonary arterial pressure
Arterial pressure

ABSTRACT

The peptide apelin is expressed in the pulmonary vasculature and is involved in the pathogenesis of many cardiovascular diseases. It has a biphasic role in the regulation of vasomotor tone related to the vascular endothelium. In this study, we induced acute pulmonary embolism (APE) in dogs with autologous blood clots to assess the effect of apelin on pulmonary and systemic circulation in the acute phase of APE. The expression of apelin mRNA was found to be upregulated in the lung tissue in the early several hours after APE induction and decreased at 24 h. The expression of apelin protein in the pulmonary arteries did not change within 24 h after APE, but significantly increased in the bronchial epithelial cells as early as 1 h and decreased at 24 h. In normal anesthetized dogs, intravenous bolus administration of apelin significantly reduced the mean arterial pressure (MAP), but did not significantly affect the mean pulmonary arterial pressure (MPAP). In the dogs with APE, apelin decreased MPAP, whereas its impact on MAP was not significantly different from that in the control group. Taken together, the level of endogenous apelin did not change significantly in the pulmonary arterial wall, whereas its expression in the bronchial epithelium was upregulated in the early stage of APE. The effect of exogenous apelin on vasomotor tone was complicated: it resulted in differential changes in the pulmonary and systemic arterial pressures under different physiological and pathological conditions.

1. Introduction

Apelin is a newly identified endogenous multifunctional peptide ligand that binds to APJ, a G protein-coupled receptor. Apelin and APJ genes are expressed extensively in body tissues and the structure of apelin is highly conserved across species [3,24]. Recently the apelin–APJ system has been reported to be involved in the pathogenesis of many common diseases such as heart failure, hypertension, atherosclerosis, diabetes mellitus type 2, and ulcerous colitis [18]. Initial experiments show that the apelin–APJ system plays a role in cardiovascular homeostasis, acting as a powerful inotropic, peripheral vasodilator and fluid homeostasis regulator [5]. However, the exact role of apelin–APJ system in cardiovascular physiology and pathology has not been completely elucidated.

Apelin is reported to have a biphasic effect on vasomotion. Vasodilation caused by apelin is endothelium-dependent. When apelin acts directly on smooth muscle cells of endothelium-denuded arteries and veins, it triggers a vasoconstrictive response [23,26]. Previous studies on the effect of apelin on blood pressure have yielded controversial results. In normotensive animals, intravenous injection of apelin lowers arterial pressure in the anesthetized state [4,9,10,17,25]. However, there is a dramatic variation in the conscious state with systemic arterial pressure being increased, decreased, not affected, or biphasically changed [1,7,16,30]. There is a difference in the methods used in different studies. It is still unclear how apelin affects arterial pressure in various disease states. In spontaneously hypertensive rats (SHRs), the expressions of apelin and APJ in the aortic tissues were markedly decreased [15,32]. The intravenous administration of apelin in SHRs significantly reduced the systemic arterial pressure [10,12]. Apelin mRNA levels have been reported to be upregulated in the ischemic myocardium in vitro after 35 min of coronary occlusion and in vivo after a 24-h ischemic period [20,29]. Apelin and APJ are found in vascular smooth muscle cells and endothelium in the lung tissue [6,9,21,24]. Recently, several studies have studied the prophylactic role of apelin in chronic pulmonary hypertension and the in vitro effect of apelin on pulmonary vasomotion [2,22]. However, the in vivo pharmacological activity of apelin on pulmonary arterial pressure has not yet been explored. Whether endogenous apelin–APJ

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doi:10.1016/j.peptides.2010.06.004

system participates in acute regulation of vascular tone is unknown.

Acute pulmonary embolism (APE) is a major cause of cardiovascular morbidity and mortality. Acute right heart failure due to the abrupt increase in pulmonary vascular resistance (PVR) is the principal cause of death in this disease. The increase in PVR after APE results from the interaction of at least three main factors that contribute to the pathophysiology of APE: mechanical obstruction of pulmonary vessels, the vasoconstriction of neurogenic pulmonary vessels, and the release of a variety of vasoconstrictors [11,27]. Although the current treatment of APE is focused on removing the mechanical obstruction in the pulmonary vessels, the accumulating experimental evidence suggests that pharmacological blockade of pulmonary vasoconstriction attenuates the hemodynamic changes associated with APE [6,11,14,27].

The purpose of the present study was to investigate whether endogenous apelin–APJ system in the pulmonary arterial wall participates in the acute regulation of the pulmonary vascular tone and to assess the effect of exogenous apelin on pulmonary arterial pressure and pulmonary vascular resistance. We induced APE in dogs with autologous blood clots and evaluated the expression of endogenous apelin–APJ in the lung tissue within 24 h after the induction of APE. We also continuously measured pulmonary and systemic hemodynamic parameters and respiratory parameters after the intravenous injection of apelin under normal anesthetized and APE conditions.

2. Methods

2.1. Animal model and hemodynamic measurement

All animal care and experimental protocols complied with the PR China Animal Management Rule (Ministry of Health, PR China) and the Animal Care Committee of the First Hospital, Harbin Medical University.

30 mongrel dogs (18 ± 2 kg) of either sex were anesthetized with ketamine (10–15 mg kg^{-1}, im) and xylazine (1.5 mg kg^{-1}, im), and maintained with ketamine (3 mg kg^{-1}, im) and xylazine (0.3 mg kg^{-1}, im) every 30 min. After tracheal intubation, the animals were ventilated with room air. Fluid-filled catheters were placed into the left femoral artery and left femoral vein for monitoring mean arterial pressure (MAP) via a pressure transducer and fluid administration, respectively. A CCO/CEDV/Svo2 774HF75 type volumetric thermodilution pulmonary artery catheter (Edwards Lifesciences, Irvine, USA) was placed into the pulmonary artery via the right femoral vein, and its correct location was confirmed by detecting of the typical pressure wave of this artery. The catheter was connected to a viglance™ monitor (Edwards Lifesciences, Irvine, USA) for measuring the continuous cardiac output. The catheter was also connected to pressure transducers to allow the monitoring of mean pulmonary artery pressure (MPAP), central venous pressure (CVP), and pulmonary capillary wedge pressure (PCWP). The transducers were zeroed at the level of the right heart and recalibrated before each set of measurements. The heart rate (HR) was measured using a surface electrocardiogram (Lead II) (Philips, Eindhoven, Netherlands).

Before the initiation of the experiments, blood samples were drawn from the femoral artery for measuring arterial oxygen tension (PaO_2), artery oxygen saturation, carbon dioxide tension ($PaCO_2$), pH, and hemoglobin using a blood gas analyzer. These respiratory parameters were within the physiological limits in all the dogs.

A 100 ml sample of the venous blood collected before the baseline measurement was allowed to clot in a glass container for 120 min and cut into 5–10 mm cubes. Next, 200 ml of saline was intravenously administered into the dogs for recovery of blood volume.

After at least 20 min of stabilization, a baseline time point hemodynamic evaluation was performed, and the animals were randomly assigned to one of the six experimental groups ($n = 5$, each group). In order to determine the effect of apelin on normal pulmonary circulation, we intravenously injected apelin-13 peptide (synthesized by GenScript Corporation, New Jersey, USA) into the dogs at two doses: 10 and 20 μg/kg in 10 ml saline, given over 30 s. Equivalent volume of saline solution was administrated to the dogs in the control group. To determine whether the intravenous injection of apelin had the same effect on hypertensive pulmonary circulation, we induced APE by repeatedly injecting the clots into the left femoral vein via a large bore polyethylene cannula at every 30 s. Embolism was progressively induced until MPAP reached 45 mmHg. Hemodynamic evaluation was performed at 30 and 60 min after the APE was induced, and the dogs in the APE-control and APE-apelin (10 or 20 μg/kg) groups received intravenous injection of saline or apelin-13, respectively, as mentioned above. The hemodynamic data and heart rate were recorded every 1 min for 10 min after apelin or saline was injected. The dose of apelin and measurement interval selected in the present study was decided on the basis of previous reports [1,7,17]. The systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated by using standard formulas.

Blood samples were drawn from the femoral artery at 1 h after APE induction and at 3 min after the injection of apelin for blood gas analysis.

2.2. Quantitative real-time polymerase chain reaction

Total RNA was extracted with TRIZOL reagent (TaKaRa, Dalian, China) following the manufacturer's instruction. Next, the RNA samples were used as templates for cDNA synthesis by using reverse transcriptase (TaKaRa, Dalian, China), Oligo dT primers and random 6 mers (TaKaRa, Dalian, China).

The expression of mRNA was determined using an ABI PRISM 7300 Real-Time PCR System (Applied Biosystems Inc, USA) and SYBR Premix Ex Taq™ (Perfect Real Time) (TaKaRa, Dalian, China). The sequences of the primers were as follows: apelin forward 5′-TCCTGCTGCTCTGGCTCTC-3′ and apelin reverse 5′-ACCAGGTGGCGGATATTGC-3′; APJ forward: 5′-GCTACTTCTTC-ATCGCCCAAA-3′ and APJ reverse: 5′-AAGGTCACCACCAGCACCA-3′; β-actin forward: 5′-TGGACTTCGAGCAGGAGATG-3′ and β-actin reverse: 5′-CAGGAAAGAAGGTTGGAAGAGTG-3′. The reaction mix contained SYBR Premix Ex Taq™, 12.5 μl; forward primer, 0.8 μl; reverse primer, 0.8 μl; cDNA, 2 μl; ROX Reference Dye, 0.5 μl; and dH2O, 8.4 μl. The thermal cycling conditions were as follows: 95 °C for 10 s, followed by 40 cycles of 95 °C for 5 s, and 62 °C for 34 s. All PCR reactions were performed in duplicate. Melting curve analysis after the amplification confirmed the specificity of the primers by yielding a single PCR product.

2.3. Immunohistochemistry

The dogs were killed at 0 (control group), 1, 8, and 24 h after APE induction (each group, $n = 5$). The lung tissues were removed, immersed and fixed overnight in 4% paraformaldehyde in buffered sodium phosphate, pH 7.0. Following paraffin processing through increasing gradients of ethanol and xylene, Paraffin embedded tissue blocks were sectioned at 4 μm, mounted on slides, and baked overnight at 60 °C. The sections were deparaffinized and rehydrated. The slides were treated in a microwave oven for 20 min in ethylenediaminetetraacetic acid (EDTA) solution (pH, 8.0) for antigen retrieval and incubated with 3% H_2O_2 for 10 min to suppress endogenous peroxidase activity. The sections were rinsed three