

Adrenomedullin alleviates not only neointimal formation but also perivascular hyperplasia following arterial injury in rats

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Abstract

Producing components of the extracellular matrix, the vascular adventitia has been recognized as an important modulator of the vascular remodeling process, which determines the vessel architecture. In this study, we examined the effect of the vasodilator peptide adrenomedullin on vascular remodeling induced by balloon injury of rat carotid arteries. Endothelial denudation with wall stretch by ballooning not only induced neointimal formation accompanied with a reduced ratio of the lumen to vessel area, but also increased the fibroblast number and collagen deposition in the adventitial layer. When compared with the saline infusion, intravenous adrenomedullin infusion at 200 ng/h for 14 days suppressed the neointimal formation (−33%, $P=0.033$), reversing the ratio of lumen to vessel ratio ($P=0.030$), without affecting systolic blood pressure. Moreover, the adrenomedullin infusion decreased the number of adventitial fibroblasts (−41%, $P<0.001$) and the collagen deposition (−36%, $P=0.006$) in the adventitial layer of the injured artery. In conclusion, the intravenous adrenomedullin infusion effectively attenuates vascular remodeling following the arterial injury via suppression of hyperplasia in the intima and adventitia, suggesting a potential of adrenomedullin as a therapeutic tool against vascular remodeling.

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1. Introduction

Arterial remodeling is a physiological and pathological reaction in response to hemodynamic, immunologic, and biochemical stimuli (Pasterkamp et al., 2004). Medial hypertrophy and neointimal lesion were focused on as important features; however recent studies have concentrated on reorganization of the entire vessel architecture as vascular remodeling (Strauss and Rabinovitch, 2000; Ward et al., 2000). Accumulating evidence suggests an importance for the adventitial layer, which modulate the remodeling process through regulation of the extracellular

matrix formation (Sartore et al., 2001; Strauss and Rabinovitch, 2000). A rodent model of arterial balloon injury is widely used to examine the remodeling process due to its similarity to restenotic vascular lesions seen after angioplasty in humans (De Meyer and Bult, 1997). In this model, the vascular injuries cause proliferation and migration of vascular smooth muscle cells (VSMC) into the intima, and fibroblasts increase in cell number, along with an increase in extracellular matrix deposition in the adventitial layer, further aggravating vascular remodeling (Sartore et al., 2001; Ryan et al., 2003). Various humoral interactions between growth factors, inflammatory cytokines or vasoactive peptides have been reported to be involved in the remodeling process (Sartore et al., 2001). Adrenomedullin, initially isolated from human pheochromocytoma (Kitamura et al., 1993), has been shown to have multiple functions in the cardiovascular system (Kitamura

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et al., 2002). Adrenomedullin was shown to inhibit the migration and proliferation of VSMC in vitro (Kano et al., 1996; Kohno et al., 1997), and Agata et al. (2003) reported that adrenomedullin gene delivery produced an inhibitory action on neointima formation after balloon injury, suggesting an important role for this bioactive peptide in vascular remodeling. However, it remains unknown whether the adrenomedullin actions are observed only in the vascular intimal layer or in the whole vascular structure in the remodeling process. The aim of the present study was to examine the biological actions of adrenomedullin on vascular remodeling, which includes not only the neointima formation but also the adventitia hyperplasia in balloon-injured carotid arteries of rats.

2. Materials and methods

The present study was performed in accordance with the Animal Welfare Act and with approval of the University of Miyazaki Institutional Animal Care and Use Committee (2003-023).

2.1. Experimental protocol

Ten- to eleven-week-old male Sprague-Dawley rats (CLEA, Japan, Inc.) weighing 350–400 g were housed in a temperature- and light-controlled room (25 ± 1 °C; 12/12-h light/dark cycle) with normal rat chow and water given ad libitum. After the rats were anesthetized with 40 mg/kg i.p. of pentobarbital sodium, endothelial denudation and wall stretch of the left common carotid artery were carried out by three passages of a Fogarty 2F balloon catheter (Baxter International, Deerfield, IL, USA). Then, the rats were randomly divided into two groups infused with saline ($n=9$) or with synthetic rat adrenomedullin (Peptide Institute, Osaka, Japan) at 200 ng/h ($n=6$) over 14 days. Immediately after the balloon injury, miniosmotic pumps (Alzet model 2002) were implanted subcutaneously to release either saline or adrenomedullin into the right external jugular vein. Blood pressure was monitored by tail-cuff plethysmography during the experimental period. At day 14, the rats were anesthetized with 40 mg/kg i.p. of pentobarbital sodium and blood samples were collected from the inferior vena cava. Both the injured left common carotid artery and non-injured contralateral were perfused via the left ventricle with phosphate buffer-saline, followed by perfusion fixation with 4% paraformaldehyde, at the physiological constant pressure of about 100 mm Hg, and were then immediately excised.

2.2. Histology and morphological evaluation

The carotid arteries embedded in paraffin were sectioned at 2 μ m thickness. After deparaffinization with xylene and graded alcohol, slides were incubated with 0.1% picrosirius red (Direct Red 80, Sigma) dissolved in

saturated picric acid for 10 min. Morphological evaluation of the injured and contralateral uninjured carotid arteries was performed at the middle portion of the artery by a single observer in a blind manner. Two samples were too disfigured to be precisely quantified: one was an injured artery of the control and the other was an intact artery of the adrenomedullin group. Therefore, these two samples were excluded from the analysis. The cross-sectional areas of the lumen and those circumscribed by the internal or external elastic lamina were determined by computerized measurement (Axio Vision 2.05 Carl ZEISS, Munchen, Germany), and the areas of the media and intima were calculated by subtraction. The vessel area was defined as the area surrounded by the external elastic lamina. The number of fibroblasts showing a typical spindle shape in the adventitia was determined at a magnification of $\times 400$. To quantify collagen deposition in the vascular wall, sections stained with picrosirius red were scanned by Mac Scope (v. 2.3.2) software under polarized light. The tightly packed collagen surrounding the carotid artery was defined as the collagen deposition in this study.

2.3. Assay for adrenomedullin

Plasma concentrations of rat adrenomedullin were measured with a specific radioimmunoassay, which detects the C-terminal amide structure of adrenomedullin, an essential portion for the biological activity, as previously described (Tsuruda et al., 1999).

2.4. Statistical analysis

All data are expressed as means \pm S.E.M. Comparisons between groups were made with one-way analysis of variance followed by the Fisher's test, and statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Plasma level of rat adrenomedullin and blood pressure

The adrenomedullin-supplemented rats showed significantly higher rat adrenomedullin levels in the plasma compared with those administered with saline at day 14 (adrenomedullin group, 4.9 ± 0.5 ; saline group, 3.3 ± 0.2 fmol/ml; $P=0.004$). Meanwhile, no significant difference in systolic blood pressure was noted before and during the experiment period (data not shown).

3.2. Effects adrenomedullin on neointimal formation and adventitia hyperplasia

Fig. 1 illustrates the hematoxylin-eosin stainings of the intact and balloon-injured carotid arteries at day 14. In the injured artery (B), neointima formation occurred and the

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