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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.

Keywords: Remodeling; Extracellular matrix; Adventitia

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 μm 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 Vison 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 ×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±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

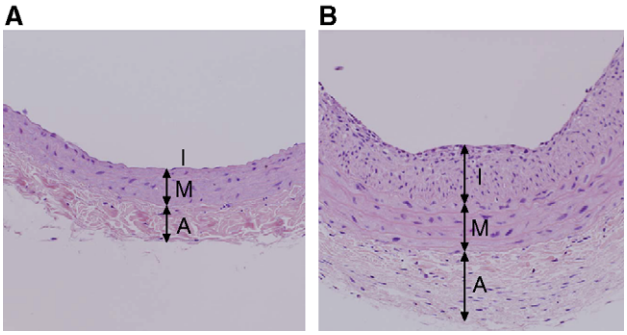

Fig. 1. Histological findings of the intact (A) and injured (B) arteries. I, intima; M, media; A, adventitia. Original magnification, ×200.

adventitial layer thickened with high cellularity, compared with the intact artery (A).

In the quantitative analysis (Fig. 2), the injured arteries showed significant neointimal formation (P<0.001) with little influence on the medial area (A) resulting in a significant increase of the intima to media ratio (B). As shown, the adrenomedullin infusion for 14 days significantly attenuated the neointimal formation by 33% (P=0.033) and the intima to media ratio by 34% (P=0.025), respectively, compared with the saline infusion, while adrenomedullin had no effect on these parameters in the contralateral, intact artery.

Fig. 3A illustrates the effect of adrenomedullin on cell number of fibroblasts in the adventitial layer. The arterial injury increased the number of fibroblasts (P<0.001), but this increase was suppressed by the adrenomedullin infusion by 41% (P<0.001). Fig. 3B shows the effect of adrenomedullin on the ratio of collagen deposition to the medial areas in the intact and injured arteries. The balloon injury enlarged the collagen deposition area mainly in the adventitia (P<0.001); however, the adrenomedullin infusion reduced it by 38% (P=0.006).

3.3. Effect of adrenomedullin on geometrical changes in the carotid arteries

Fig. 4A and B illustrate the effect of adrenomedullin on the lumen and vessel areas, respectively. The balloon injury slightly reduced the lumen area of rats infused with saline,

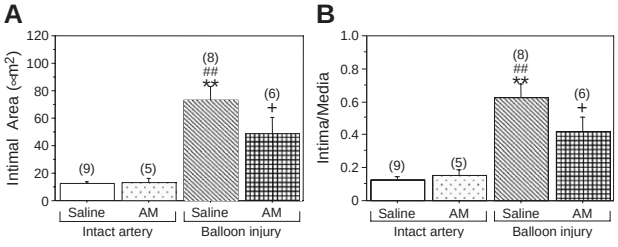

Fig. 2. Effect of adrenomedullin on intimal area (A) and ratio of intima to media (B) in the intact and injured arteries. Values are means±S.E.M., (n). **P<0.01 vs. intact artery with saline infusion; $^{\#\#}P$<0.01 vs. intact artery with adrenomedullin infusion; ^{+}P<0.05 vs. injured artery with saline infusion.

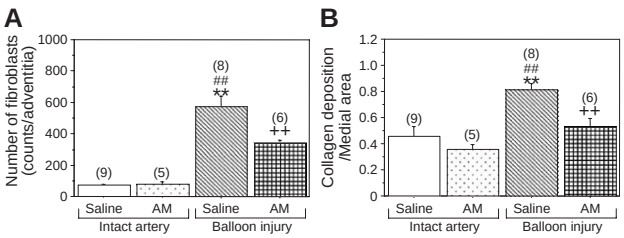

Fig. 3. Effect of adrenomedullin on cell number in the adventitia (A) and collagen deposition/medial area (B). Values are means±S.E.M., (n). **P<0.01 vs. intact artery with saline infusion; $^{\#\#}P$<0.01 vs. intact artery with adrenomedullin infusion; ^{++}P<0.01 vs. injured artery with saline infusion.

but this reduction was statistically insignificant (Fig. 4A), and no significant differences were noted in the vessel area of four study groups (Fig. 4B). As shown in Fig. 4C, the ratio of the lumen to vessel area was significantly (P<0.001) reduced by the balloon injury in the saline group, compared with those of the intact arteries. The adrenomedullin supplement significantly (P=0.030) reversed this geometrical change toward those of the intact arteries.

4. Discussion

We report here that intravenous adrenomedullin infusion not only attenuated neointima formation but also inhibited fibroblast proliferation and collagen deposition of the adventitia, reducing the ratio of lumen to vessel area, in the balloon-injured carotid arteries of rats. The three layers of the vascular wall, intima, media and adventitia, contribute to inward or outward remodeling which occurs following arterial injury (Ward et al., 2000). Although neointimal formation and medial hypertrophy have been focused on as targets in preventing adverse remodeling, recent reports have

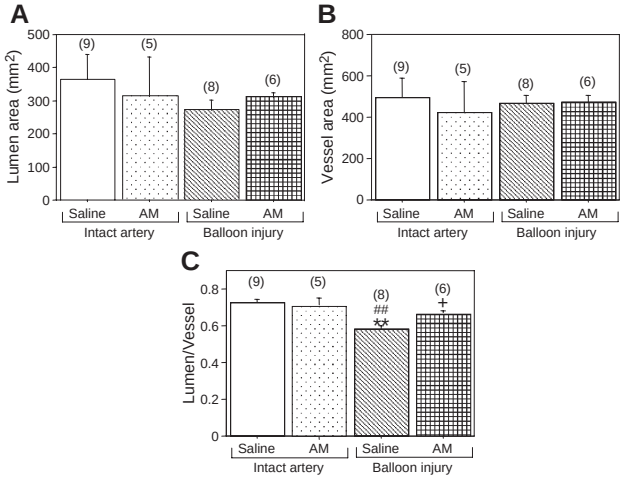

Fig. 4. Effect of adrenomedullin on lumen area (A), vessel area (B) and ratio of lumen to vessel area (C). Values are means±S.E.M., (n). **P<0.01 vs. intact artery with saline infusion; $^{\#\#}P$<0.01 vs. intact artery with adrenomedullin infusion; ^{+}P<0.05 vs. injured artery with saline infusion.

referred more to the role of the adventitial layer (Ryan et al., 2003; Sartore et al., 2001; Strauss and Rabinovitch, 2000).

In a model of arterial injury, endothelial denudation induces VSMC proliferation and migration, making up the neointima formation. Our present data supports previous studies showing that adrenomedullin attenuated the neointima formation induced by arterial injuries in rats (Agata et al., 2003; Yamasaki et al., 2003) and in mice (Imai et al., 2002; Kawai et al., 2004). On the other hand, extracellular matrix deposition in the adventitia with cellular hyperplasia appears to be the major phenomena responsible for adventitial thickening that would subsequently increase stiffness of the vascular walls and peripheral arterial resistance (Intengan and Schiffrin, 2001; Sartore et al., 2001). Importantly, we found that the adrenomedullin administration significantly decreased the number of fibroblasts in the adventitia following the arterial injury in this study. In addition, the adrenomedullin-treated rats showed a significant reduction of collagen deposition in the entire vessel wall, mainly in the adventitia. Considering the importance of extracellular matrix formation in determining stiffness of the vascular wall (Intengan and Schiffrin, 2001), adrenomedullin may exert a beneficial action alleviating vascular stiffness.

In this study, the beneficial effects of adrenomedullin following arterial injury were observed without a significant effect on blood pressure, suggesting a direct action of adrenomedullin on the vascular remodeling. Adrenomedullin has been shown to directly inhibit proliferation and migration of cultured VSMC (Kano et al., 1996; Kohno et al., 1997), and according to our previous report (Tsuruda et al., 1999), adrenomedullin inhibited proliferation of cultured fibroblasts isolated from rat cardiac ventricle. Recently, we reported that adrenomedullin induced matrix metalloproteinase-2 activity in cultured adventitial fibroblasts isolated from rat aorta (Tsuruda et al., 2004). Collagen accumulation is responsible for constrictive remodeling following balloon injury (Ryan et al., 2003). Proteolytic activity induced by adrenomedullin may have contributed to attenuating collagen deposition, however these hypotheses for possible, direct actions of adrenomedullin should be tested in vivo by future experiments.

In summary, the intravenous adrenomedullin infusion effectively improves the vascular geometry of the balloon-injured rat carotid artery, suppressing neointima formation, adventitial fibroblast proliferation and collagen deposition. This study implies a possible utility of adrenomedullin for inhibition of vascular remodeling, where both neointimal formation and adventitial hyperplasia are targeted.

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References

Agata, J., Zhang, J.J., Chao, J., Chao, L., 2003. Adrenomedullin gene delivery inhibits neointima formation in rat artery after balloon angioplasty. Regul. Pept. 112, 115–120.

De Meyer, G.R., Bult, H., 1997. Mechanisms of neointima formation—lessons from experimental models. Vasc. Med. 2, 179–189.

Imai, Y., Shindo, T., Maemura, K., Sata, M., Saito, Y., Kurihara, Y., Akishita, M., Osuga, J., Ishibashi, S., Tobe, K., Morita, H., Oh-hashi, Y., Suzuki, T., Maekawa, H., Kangawa, K., Minamino, N., Yazaki, Y., Nagai, R., Kurihara, H., 2002. Resistance to neointimal hyperplasia and fatty streak formation in mice with adrenomedullin overexpression. Arterioscler. Thromb. Vasc. Biol. 22, 1310–1315.

Intengan, H.D., Schiffrin, E.L., 2001. Vascular remodeling in hypertension. Roles of apoptosis, inflammation, and fibrosis. Hypertension 38, 581–587.

Kano, H., Kohno, M., Yasunari, K., Yokokawa, K., Horio, T., Ikeda, M., Minami, M., Hanehira, T., Takeda, T., Yoshikawa, J., 1996. Adrenomedullin as a novel antiproliferative factor of vascular smooth muscle cells. J. Hypertens. 14, 209–213.

Kawai, J., Ando, K., Tojo, A., Shimosawa, T., Takahashi, K., Onozato, M.L., Yamasaki, M., Ogita, T., Nakaoka, T., Fujita, T., 2004. Endogenous adrenomedullin protects against vascular response to injury in mice. Circulation 109, 1147–1153.

Kitamura, K., Kangawa, K., Kawamoto, M., Ichiki, Y., Nakamura, S., Matsuo, H., Eto, T., 1993. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem. Biophys. Res. Commun. 192, 553–560.

Kitamura, K., Kangawa, K., Eto, T., 2002. Adrenomedullin and PAMP: discovery, structures, and cardiovascular functions. Microsc. Res. Tech. 57, 3–13.

Kohno, M., Yokokawa, K., Kano, H., Yasunari, K., Minami, M., Hanehira, T., Yoshikawa, J., 1997. Adrenomedullin is a potent inhibitor of angiotensin II-induced migration of human coronary artery smooth muscle cells. Hypertension 29, 1309–1313.

Pasterkamp, G., Galis, Z.S., de Kleijn, D.P., 2004. Expansive arterial remodeling: location, location, location. Arterioscler. Thromb. Vasc. Biol. 24, 650–657.

Ryan, S.T., Koteliansky, V.E., Gotwals, P.J., Lindner, V., 2003. Transforming growth factor-beta-dependent events in vascular remodeling following arterial injury. J. Vasc. Res. 40, 37–46.

Sartore, S., Chiavegato, A., Faggin, E., Franch, R., Puato, M., Ausoni, S., Pauletto, P., 2001. Contribution of adventitial fibroblasts to neointima formation and vascular remodeling: from innocent bystander to active participant. Circ. Res. 89, 1111–1121.

Strauss, B.H., Rabinovitch, M., 2000. Adventitial fibroblasts: defining a role in vessel wall remodeling. Am. J. Respir. Cell Mol. Biol. 22, 1–3.

Tsuruda, T., Kato, J., Kitamura, K., Kawamoto, M., Kuwasako, K., Imamura, T., Koiwaya, Y., Tsuji, T., Kangawa, K., Eto, T., 1999. An autocrine or a paracrine role of adrenomedullin in modulating cardiac fibroblast growth. Cardiovasc. Res. 43, 958–967.

Tsuruda, T., Kato, J., Cao, Y.N., Hatakeyama, K., Masuyama, H., Imamura, T., Kitamura, K., Asada, Y., Eto, T., 2004. Adrenomedullin induces matrix metalloproteinase-2 activity in rat aortic adventitial fibroblasts. Biochem. Biophys. Res. Commun. 325, 80–84.

Yamasaki, M., Kawai, J., Nakaoka, T., Ogita, T., Tojo, A., Fujita, T., 2003. Adrenomedullin overexpression to inhibit cuff-induced arterial intimal formation. Hypertension 41, 302–307.

Ward, M.R., Pasterkamp, G., Yeung, A.C., Borst, C., 2000. Arterial remodeling. Mechanisms and clinical implications. Circulation 102, 1186–1191.