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Differential modulation of nitric oxide and prostacyclin release in senescent rat heart stimulated by angiotensin II

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Abstract

To elucidate the mechanism of age-related changes in the cardiovascular function stimulated with angiotensin II, we examined the effects of angiotensin II on the coronary flow, production of nitric oxide (NO) and prostacyclin, and on the cardiac function in the Langendorff-perfused young and aged rats' hearts. Angiotensin II decreased coronary flow, left ventricular dP/dt and heart rate. These effects were more pronounced in aged rats. Pretreatment with a NO synthase inhibitor, N^G-nitro-L-arginine, significantly increased the angiotensin II-induced vasoconstriction in young rats. Angiotensin II increased the concentration of NO in the coronary effluent in young but not in aged rats. In contrast, angiotensin II stimulated the release of prostacyclin to a much greater extent in aged rats than in young rats. These results suggest that impaired production of NO may contribute to the greater constrictor effect of angiotensin II in the aged rat, although aging modulated the production of prostacyclin in a different manner. This age-related endothelial dysfunction may alter the physiological regulation of coronary flow and cardiac function stimulated with angiotensin II.

Keywords: Aging; Angiotensin II; Endothelial function; Nitric oxide (NO); Prostacyclin; Rat

1. Introduction

The renin–angiotensin system regulates vascular tone with physiological significance. Although there is a species-dependent difference in the action of angiotensin II (Ishihata and Endoh, 1995), activation of angiotensin II receptor mediates a variety of effects such as contractions and hypertrophy of cardiac and vascular smooth muscle, catecholamine release from autonomic nerve endings, aldosterone secretion from adrenal glands, adrenocorticotropic hormone (ACTH) and prolactin secretion from the anterior pituitary glands (Ishihata and Endoh, 1993, 1995; Pörsti et al., 1993). Therefore, angiotensin II is implicated in the pathogenesis and progression of various cardiovascular diseases such as hypertension, atherosclerosis and cardiac hypertrophy. The process of aging is also associated with many functional changes in the cardiovascular system. Such age-related changes may be associated with the altered cardiovascular effects of vasoactive substances like angiotensin II, endothelin and vasopressin.

Endothelium plays an important role in regulating vascular tone and in maintaining the cardiovascular function. Two of the well-known factors involved in vasodilatation are endothelium-derived nitric oxide (NO) and prostacyclin. They are released not only in the basal condition but also in response to various vasodilating substances such as bradykinin, and to the intravascular shear stress (Lamontagne et al., 1992; Katano et al., 1993). Along with vasodilating substances, a strong vasoconstrictor angiotensin II can also stimulate release of NO and prostacyclin from endothelial cells (Yamazaki and Toda, 1991).

Besides NO and prostacyclin, atrial natriuretic peptide (ANP) also plays an important role in regulating coronary circulation in vivo (Liu et al., 1996). ANP exerts vasodilating effects through increasing cGMP in vascular smooth muscle cells. In patients under severe congestive heart failure, it is reported that the endogenous ANP was increased to maintain the coronary flow as a compensatory mechanism (Ding et al., 1987; Liu et al., 1996).

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In order to elucidate the mechanism of age-related changes in the coronary circulation as well as the cardiac function stimulated with angiotensin II, we examined the effects of angiotensin II on the coronary flow and on the cardiac function in rat heart. For revealing the role of NO, prostacyclin and ANP in the age-related changes in the cardiovascular function, those factors were analyzed in the coronary effluent from young and aged rat stimulated with angiotensin II.

2. Materials and methods

In this study, 2- to 3-month-old (200–250 g) and 24- to 27-month-old (320–370 g) male Fischer 344 rats obtained from Charles River Japan (Atsugi, Japan) were used. They were maintained on standard rat chow with water ad libitum. Experiments were performed in accordance with the ''Guide for Care and Use of Laboratory Animals'' published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996) and under the regulations of the animal care committee of Yamagata University School of Medicine.

2.1. Measurement of coronary circulation and cardiac function in an isolated perfused heart

Each rat was anesthetized with ether and decapitated, and the heart was quickly removed. The isolated heart was immediately perfused by Langendorff's method under constant pressure (75 cm H_2O) at 37 ± 0.1°C with modified Krebs–Ringer bicarbonate solution of the following composition (in mM): NaCl 118, KCl 4.7, $NaHCO_3$ 24.9, $MgSO_4$ 1.18, KH_2PO_4 1.18, $CaCl_2$ 1.8, glucose 5.0, pyruvic acid 2.0, ascorbic acid 0.057. The buffer solution was continuously aerated with 95% O_2–5% CO_2 (pH 7.4). The coronary flow (ml/min) was measured with an electromagnetic flow-meter (MFV 1100, Nihon Kohden, Tokyo, Japan). The left ventricular pressure was recorded with a saline-filled intraventricular balloon connected to a pressure transducer (Statham P-50, Gould). The rate of left ventricular pressure development (dP/dt) was obtained with an electronic differentiator (EQ-601G, Nihon Kohden, Tokyo, Japan). The heart rate was detected with tachometer (AT-601G, Nihon Kohden, Tokyo, Japan) triggered by left ventricular pressure pulses. It has been demonstrated that angiotensin II has no direct negative inotropic effect in the rat papillary muscle (Ishihata and Endoh, 1995). However, in the Langendorff-perfused rat heart, angiotensin II inhibited cardiac function. In the young rat, angiotensin II reduced the maximal dP/dt to 87.3 ± 8.5% of the basal value. In the aged rat, maximal dP/dt decreased to 68.7 ± 2.3% of the basal value.

Acetylcholine (10, 30 pmol) or angiotensin II (1 pmol) was applied into the coronary artery as a bolus (< 0.1 ml) for 10 s, and N^G-nitro-L-arginine (L-NNA) was applied by continuous infusion into the rubber tubing connected to the aortic cannula. In the experimental group for the pretreatment with L-NNA, the continuous infusion of L-NNA started 10 min before and continued during the application of acetylcholine or angiotensin II (syringe pump, Harvard Apparatus 940e, Mills, MA, USA). The changes in the coronary flow were expressed as percentage of the basal flow just before the injection of acetylcholine or angiotensin II. The responses to acetylcholine and sodium nitroprusside were reversible. Because the response to angiotensin II was desensitized and not reproducible, the response to angiotensin II in the presence and absence of L-NNA was recorded in different hearts.

2.2. Measurement of the concentration of prostacyclin in the coronary effluent

The concentration of 6-keto-prostaglandin $F_{1\alpha}$ which is a stable metabolite of prostacyclin, was measured by enzyme-immunoassay. In brief, coronary effluent was seriously collected for every 1 min just before and after the administration of angiotensin II for the measurement of 6-keto-prostaglandin $F_{1\alpha}$. The concentration of 6-keto-prostaglandin $F_{1\alpha}$ in the effluent was measured by using commercially available enzyme-immunoassay kit for 6-keto prostaglandin $F_{1\alpha}$ (Cayman Chemical, Ann Arbor, MI, USA).

2.3. Measurement of the concentration of ANP

The coronary effluent was collected before starting the infusion of drugs and after infusion of them. The samples were frozen and kept at -70°C until analysis. The concentration of ANP in the coronary perfusate was measured by radio immunoassay according to the method described previously with some modification (Nishikimi et al., 1996).

Table 1
Effects of L-NNA on the coronary flow. Values are means ± S.E.M. Abbreviations: L-NNA, N^G-nitro-L-arginine; CF, coronary flow; HW, heart weight

	Heart weight (HW, g)	CF (ml/min)		Basal CF/HW (ml/min/g)		*n*
		Baseline	After L-NNA	Baseline	After L-NNA	
Young	0.92 ± 0.12	6.25 ± 0.46	5.91 ± 0.46[a]	7.20 ± 0.82	6.80 ± 0.77[a]	7
Aged	1.35 ± 0.05[b]	8.11 ± 0.95	7.7 ± 1.00[a]	5.96 ± 0.47	5.65 ± 0.54[a]	5

[a] $P < 0.05$ vs. baseline.
[b] $P < 0.05$ vs. young.

[^{125}I]-iodotyrosyl^{28} ANP (rat) (2200 Ci/mmol, New England Nuclear, USA) and rabbit antiserum to rat ANP-(1–28) (New England Nuclear, USA) were used in this radio immunoassay.

2.4. Measurement of the concentration of NO

NO secreted from the coronary artery is rapidly decomposed to more stable products, nitrate (NO_3^-) and nitrite (NO_2^-). The total amount of NO_3^- plus NO_2^- (NO_x) was determined by the method described previously (Misko et al., 1993). In brief, an aliquot of coronary effluent was incubated with nitrate reductase to reduce NO_3^- into NO_2^- (Granger et al., 1996). Then, the NO_2^- was mixed with 2,3-diaminonaphthalene (DOJINDO, Kumamoto, Japan) in an acidic condition (pH < 2) at room temperature. The reaction product (naphthalenetriazole) was measured by using a spectrofluorometer (Hitachi 650-10, Tokyo, Japan) with excitation at 365 nm and emission at 450 nm in a basic condition (pH > 10). The NO_2^- concentration was determined by interpolation of a calibration curve of standard sodium nitrate ($NaNO_3$) concentration against fluorescence intensity.

2.5. Statistical analysis

Data are expressed as means ± standard error of the mean (S.E.M). Comparisons among multiple groups were assessed by repeated measure analysis of variance (ANOVA) with pairwise comparison by Bonferroni method. Differences between two groups were analyzed using the unpaired Student's *t*-test. A *P*-value less than 0.05 was considered to be statistically significant.

2.6. Materials used

Drugs used were acetylcholine chloride, N^5-[nitroamidino]-L-2,5-diaminopentanoic acid (L-NNA, Sigma, St. Louis, MO, USA), sodium nitroprusside dihydrate (Wako Chemical, Osaka, Japan) and angiotensin II (Peptide Institute, Osaka, Japan). Drug solutions were prepared freshly on each experimental day.

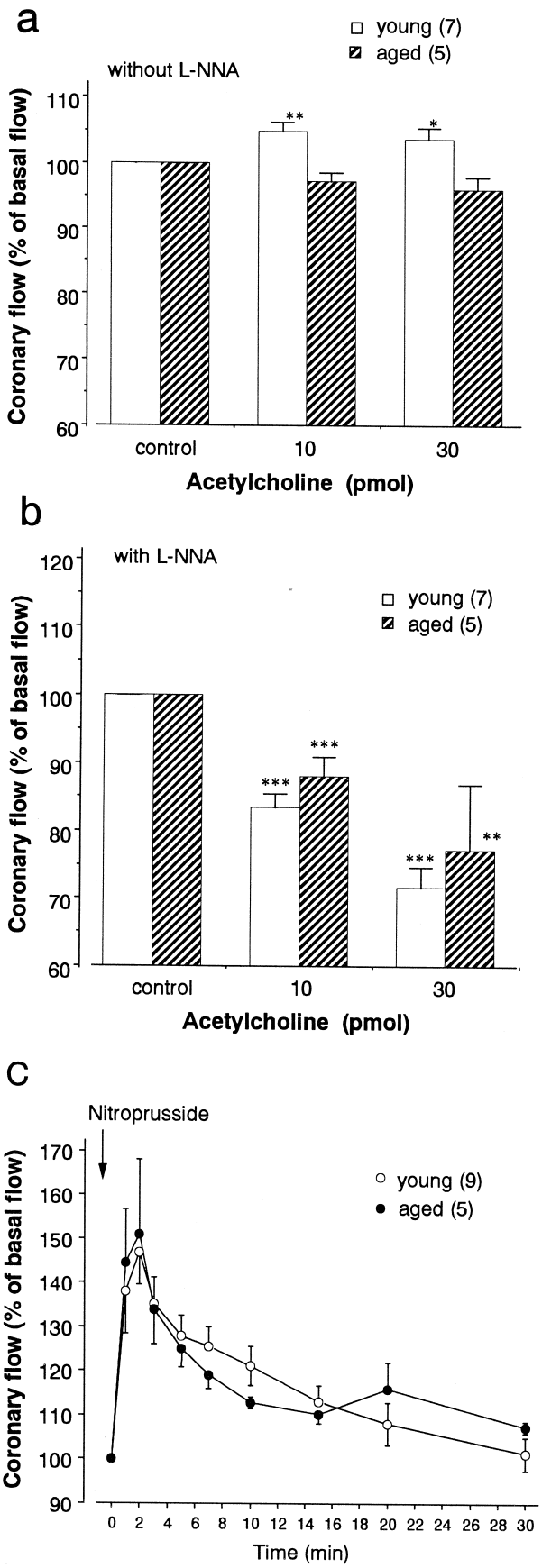

Fig. 1. Influence of age on the coronary vascular response to acetylcholine and sodium nitroprusside. (a) Effects of acetylcholine (10 and 30 pmol) in the absence of L-NNA, an inhibitor of NO synthase, in the young and aged rat. The ratio of basal coronary flow and heart weight was 7.45 ± 0.46 ml/min/g in the young rat, and 6.93 ± 0.74 ml/min/g in the aged rat. (b) Effects of acetylcholine in the presence of L-NNA (100 μM). The ratio of coronary flow and heart weight before the addition of acetylcholine was 6.80 ± 0.77 ml/min/g in the young rat, and 5.65 ± 0.54 ml/min/g in the aged rat. Coronary flow was expressed as a percentage of the basal coronary flow. (c) Effects of sodium nitroprusside (100 nmol) in the young and aged rat. The ratio of basal coronary flow and heart weight was 6.74 ± 0.89 ml/min/g in the young rat, and 7.15 ± 1.06 ml/min/g in the aged rat. Results are expressed as mean ± S.E.M. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. basal coronary flow.

3. Results

3.1. Influence of aging on the coronary vascular response to acetylcholine and nitroprusside

In order to compare the endothelial function between young and aged rat, the coronary vascular response to acetylcholine was measured. The basal value of coronary flow was 6.25 ± 0.46 ml/min in the young rat, and 8.11 ± 0.95 ml/min in the aged rat (Table 1). The heart weight of aged rat was 1.35 ± 0.05 g, and that of young rat was 0.92 ± 0.12 g. The ratio of coronary flow and heart weight (coronary flow/heart weight) was not different between each group (Table 1). Acetylcholine caused a coronary vasodilatation in the young rat, although the vasodilating effect was not obvious in the aged rat (Fig. 1a). Pretreatment with a NO synthase inhibitor, L-NNA (100 μM) decreased the basal coronary flow (Table 1). In the experimental group that was pretreated with L-NNA, acetylcholine decreased the coronary flow even in the young rat (Fig. 1b). In the aged rat pretreated with L-NNA, acetylcholine induced a coronary vasoconstriction. On the other hand, sodium nitroprusside (100 nmol) increased the coronary flow in both young and aged rat, and the vasodilating effect of nitroprusside was not altered by aging (Fig. 1c).

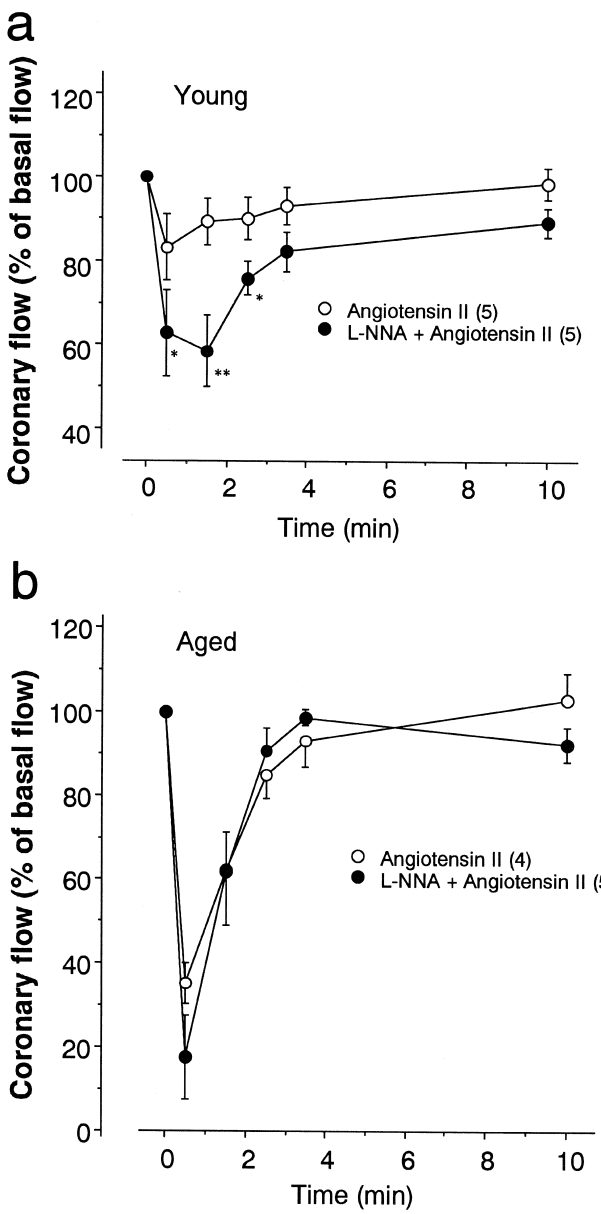

Fig. 2. Effect of angiotensin II on the coronary flow in the (a) young and (b) aged rat. Angiotensin II (1 pmol) was infused in the absence or in the presence of L-NNA (100 μM). The ratio of basal coronary flow and heart weight (CF/HW) was 7.23 ± 0.45 ml/min/g in the young rat, and 7.06 ± 0.80 ml/min/g in the aged rat in the case of angiotensin II alone. In the L-NNA pretreated group, the ratio of basal coronary flow and heart weight (CF/HW) was 6.61 ± 0.51 ml/min/g in the young rat, and 5.97 ± 0.50 ml/min/g in the aged rat. Results are expressed as mean ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. angiotensin II alone.

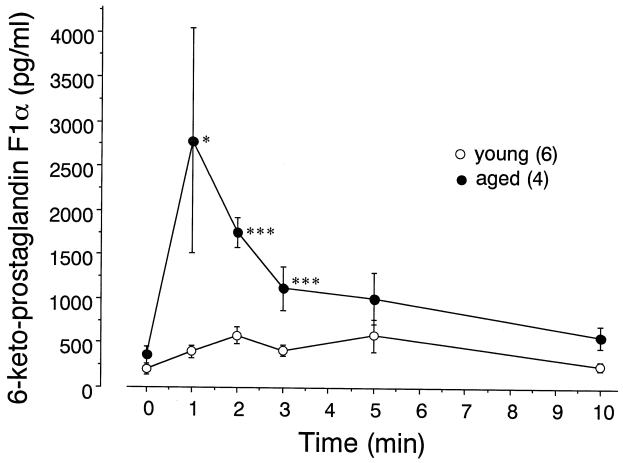

Fig. 3. Effect of angiotensin II on the concentration of prostacyclin released into the coronary effluent of young and aged rat. The concentration of prostacyclin was measured as its stable metabolite (6-keto-prostaglandin $F_{1\alpha}$) by enzyme-immunoassay. The basal value of 6-keto-prostaglandin $F_{1\alpha}$ was 202.7 ± 66.7 pg/ml in the young rat ($n = 6$), and was 362.8 ± 97.9 pg/ml in the aged rat ($n = 4$), respectively. Results are expressed as mean ± S.E.M. $^{*}P < 0.05$ vs. young rat.

3.2. Effect of angiotensin II on the coronary flow in the young and aged rat

Fig. 2 shows the effect of angiotensin II on the coronary flow in the perfused heart. In the young rat, coronary flow decreased to 83.2 ± 7.7% ($n = 5$) of the basal value at 30 s, then it gradually increased and returned to the basal level after 10 min. In the young rat pretreated with L-NNA (100 μM), angiotensin II induced a greater coronary vasoconstriction than in the young rat without pretreatment of L-NNA (Fig. 2a). In contrast, angiotensin II markedly reduced the coronary flow (35.3 ± 4.7%, $n = 4$) in the aged rat heart even in the absence of L-NNA (Fig. 2b). The angiotensin II-induced coronary vasoconstriction was not significantly affected by L-NNA, suggesting that the re-

lease of NO in the aged rat was not facilitated in response to angiotensin II (Fig. 2b).

3.3. Effects of angiotensin II on the release of prostacyclin and ANP in the coronary effluent

The basal level of 6-keto-prostaglandin $F_{1\alpha}$ in the perfusate was not significantly different (202.7 ± 66.7 pg/ml in the young rat ($n = 6$); 362.8 ± 97.9 pg/ml in the aged rat ($n = 4$)). In the aged rat, angiotensin II increased 6-keto-prostaglandin $F_{1\alpha}$ to the peak level of 2781 ± 1267 pg/ml of the basal value within a minute. Then 6-keto-prostaglandin $F_{1\alpha}$ gradually decreased to 581 ± 123 pg/ml of the basal value after 10 min. In the young rat, 6-keto-prostaglandin $F_{1\alpha}$ increased to 400 ± 66 pg/ml of the basal value at 1 min after addition of angiotensin II (Fig. 3).

In the coronary effluent, the basal level of ANP was 1.25 ± 0.34 fmol/ml ($n = 5$) in the young rat, while it tended to be higher (1.71 ± 0.08 fmol/ml) in the aged rat ($n = 4$). Angiotensin II increased the concentration of ANP to 2.43 ± 1.06 fmol/ml in the young rat and to 4.92 ± 1.21 fmol/ml in the aged rat (Fig. 4).

3.4. Effects of angiotensin II on the release of NO into the coronary perfusate

The concentration of NO_x in the coronary perfusate was increased rapidly by the administration of angiotensin II in the young rat. The basal value of NO_x was 0.59 ± 0.10 μM in the young rat ($n = 6$) and 0.50 ± 0.23 μM in the aged rat ($n = 4$), respectively. The level of NO_x in the young rat increased to several-fold of the basal level after injection of angiotensin II. In the aged rat, however, the increase in the production of NO_x in response to angiotensin II was significantly lower than that in the young rat (Fig. 5). The coronary NO_x in the aged rat increased but not significantly after administration of angiotensin II, and the response was short-lasting.

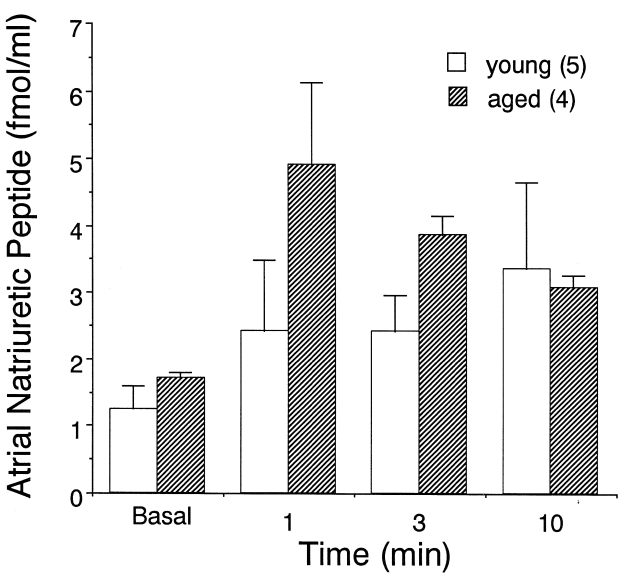

Fig. 4. Effect of angiotensin II on the concentration of ANP released into the coronary effluent of young and aged rat. The concentration of ANP was measured by radio-immunoassay. The basal value of ANP was 1.25 ± 0.34 fmol/ml in the young rat ($n = 5$) and 1.71 ± 0.08 fmol/ml in the aged rat ($n = 4$), respectively. Results are expressed as mean ± S.E.M.

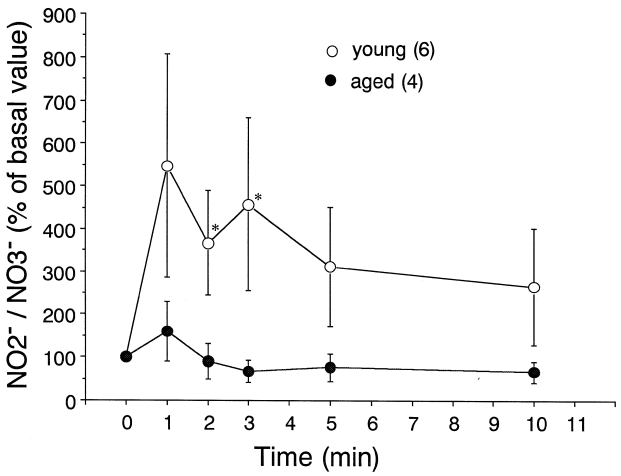

Fig. 5. Effect of angiotensin II on the concentration of NO produced in the young and aged rat coronary artery. After infusion of angiotensin II, the concentration of nitrate plus nitrite (NO_x) in the coronary effluent was measured fluorometrically. The basal value of NO_x was 0.59 ± 0.10 μM in the young rat ($n = 6$) and 0.50 ± 0.23 μM in the aged rat ($n = 4$), respectively. Results are expressed as mean ± S.E.M. $^*P < 0.05$ vs. aged rat.

4. Discussion

In this study, we have demonstrated that angiotensin II stimulated the release of NO in the coronary artery of young rat, but the production was attenuated in the aged rat. The aged coronary endothelial cells could not release as much amount of NO as the young endothelial cells in response to a strong vasoconstrictor, angiotensin II. This endothelial dysfunction may induce the strong coronary vasoconstriction in the aged rat. In fact, angiotensin II reduced the coronary flow to 83% of the basal value in the young rat, while it strongly inhibited the coronary flow of aged rat to 35%. On the other hand, angiotensin II decreased dP/dt max to 87% of the basal value in the young rat, and to 69% in the aged rat. Therefore, a greater inhibition of coronary flow than max dP/dt in the aged rat suggested that angiotensin II could reduce the coronary flow by a mechanism that was not necessarily relevant to the changes in the cardiac function. In our constant pressure Langendorff preparation, angiotensin II decreased the heart rate and the effect was more pronounced in the aged rat. It is possible that the decreased heart rate by angiotensin II reduced extravascular coronary resistance to

cause variations of coronary flow. Similarly, acetylcholine transiently decreased the heart rate in the Langendorff preparation, therefore coronary flow may have been affected by heart rate in addition to the alteration of endothelial NO formation (Fig. 1). In contrast to the diminished production of NO, the aged coronary artery could produce a higher level of prostacyclin. These findings indicate that aging may modulate the release of NO and prostacyclin in a different manner.

The coronary vasomotion is regulated by a variety of factors such as NO, prostacyclin, endothelium-derived hyperpolarizing factor, endothelin and other endothelium-derived vasoconstrictor factors (Bassenge, 1995). Both prostacyclin and NO are reported to contribute to the vasodilating effects of bradykinin in the perfused rabbit heart (Lamontagne et al., 1992). In addition, such vasoconstricting substances as endothelin or angiotensin II have been shown to stimulate the release of prostacyclin and/or NO in dog mesenteric artery, rat coronary artery and pregnant sheep uterine artery (Yamazaki and Toda, 1991; Katano et al., 1993; Magness et al., 1996).

In this study, while the coronary vasodilating effect of sodium nitroprusside in the aged rat was comparable with that of the young rat (Fig. 1c), acetylcholine did not dilate coronary artery in the aged heart (Fig. 1a). These results suggest that the responsiveness of smooth muscle of coronary artery to NO was not diminished by aging. However, potential role of a membrane-bound NADH oxidoreductase in NO release and arterial relaxation by nitroprusside has been reported in bovine pulmonary artery (Mohazzab-H et al., 1999) and in coronary arterial smooth muscle (Elizabeth et al., 1992), although nitroprusside was at first believed to release NO spontaneously (Ignarro et al., 1981) and widely used to estimate the smooth muscle responsiveness to NO (Küng and Lüscher, 1995). Therefore, it should be important for a strictly precise evaluation of the responsiveness of smooth muscle to NO to examine the NADH oxidoreductase activity and the actual amount of NO released by nitroprusside in each heart. In contrast to the apparently identical responsiveness of smooth muscle to NO, the ability to release NO in the endothelium was suggested to be impaired by aging. These findings are consistent with the previous reports of ours and others, in which an impaired response to acetylcholine with aging was observed in the coronary artery of humans and rats. Amrani et al. (1996) also reported that aging was associated with reduced basal release and 5-hydroxytryptamine-stimulated release of NO. Furthermore, numerous studies suggested that a variety of pathophysiological conditions with the coronary artery diseases were associated with the impaired production of NO. For example, NO-dependent vasodilatation was attenuated in hyperlipidemia, diabetes and chronic heart failure (Katano et al., 1993; Chauhan et al., 1996; Dusting, 1996; Lyons, 1997). We have shown here that aging as well is one of the pathophysiological status of impaired production of NO.

There may exist a difference in the time-course of endothelial impairment among vessels. In 18- to 24-month-old rat aorta, endothelium-dependent vasorelaxation is reported to be unchanged or only a little impaired (about 20% inhibition in acetylcholine-induced maximal relaxation) compared with young rat aorta (Küng and Lüscher, 1995; Engler and Engler, 1996). In 32- to 33-month-old rat aorta, endothelium-dependent vasorelaxation as well as endothelial NO production was strongly inhibited (Tschudi et al., 1996). In our present study, endothelial function of Langendorff-perfused heart of 24–27-month-old rat was shown to be already impaired. Because changes in coronary flow in Langendorff preparations reflect the tone of the microcirculation, these findings imply that aging, in contrast to other causes of endothelial dysfunction such as hypertension (Küng and Lüscher, 1995), affects endothelial function of the microcirculation prior to that of large arteries, although these results were possibly affected by differences in the rat used in each study (male Fischer 344, WKY and female RORO rat).

The endothelium-derived vasorelaxing factors may play a role in counteracting the vasoconstriction induced by such factors as angiotensin II and endothelin-1 (Katano et al., 1993). In this study, we showed that the concentration of NO in the coronary effluent increased in response to angiotensin II in the young rat. However, little increase was observed in the aged rat (Fig. 5). Although the production of NO was less in the aged rat, it should be noticed that the concentration of prostacyclin increased to a much greater extent in the aged rat coronary effluent than in the young rat (Fig. 3). It might be possible that aged endothelial cells could produce prostacyclin as a compensation-mechanism for reduced production of NO. However, it is not clarified yet from our present study whether the released prostacyclin could play a compensatory role in the aged rat.

ANP modulates blood pressure through activating membrane-type guanylate cyclase of smooth muscle cells (Brenner et al., 1990). ANP is secreted primarily in the atrial myocytes by the atrial stretch (Dietz, 1987) as well as by many hormones such as vasopressin, acetylcholine, endothelin and angiotensin II (Sonnenberg and Veress, 1984; Schiebinger and Gomez, 1990; Volpe et al., 1990). We showed here that the concentration of ANP tended to be higher in the aged rat than in the young rat. Because the angiotensin II-induced coronary vasoconstriction and the inhibition of cardiac function may in turn increase the cardiac filling pressure to stretch the cardiac wall, the release of ANP would be more pronounced. There is another hypothesis for the regulation of the ANP-production that the increased NO may negatively modulate the ANP-secretion in the heart (Sagnella et al., 1986; Ding et al., 1987; Sanchez et al., 1990; Ontkean et al., 1991; Buckley et al., 1993; Melo and Sonnenberg, 1996).

It is of interest that one previous report suggested that the half of the effect of the angiotensin II-induced vaso-

constriction and the release of prostacyclin could be mediated by the released endothelin-1 (Oriji and Keiser, 1997). The production of endothelin-1 is inhibited by NO and ANP through increased formation of cGMP (Noll et al., 1996). Therefore, it might be possible that the angiotensin II-induced strong coronary vasoconstriction in the aged heart could be mediated by endothelin-1. On the contrary, angiotensin II had a lesser vasoconstriction in the young because the much more released NO could inhibit the production of endothelin-1. However, this hypothesis may not be probable, because the vasoconstriction caused by angiotensin II was short-lasting and rapidly desensitized, and absolutely different from the time-course of endothelin-1-induced vasoconstriction (Katano et al., 1993).

In conclusion, this study revealed that angiotensin II induced coronary vasoconstriction to a much greater extent in the aged rat because of the endothelial dysfunction, especially in the production of NO. The impairment of the endothelial function may alter the concerted regulation of coronary flow and may be responsible for the negative cardiac effects of angiotensin II in the aged rat. In contrast, the aged coronary artery was shown to produce prostacyclin to a greater extent than young coronary artery, indicating that aging modulates the release of NO and prostacyclin in a different manner. Although the detailed mechanisms for the age-related diminished production of NO and for the increased releases of prostacyclin remain to be elucidated, the present study suggests that the modulation by aging of the regulation of coronary circulation may have an important role in the altered cardiovascular effect of angiotensin II.

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References

Amrani, M., Goodwin, A.T., Gray, C.C., Yacoub, M.H., 1996. Ageing is associated with reduced basal and stimulated release of nitric oxide by the coronary endothelium. Acta Physiol. Scand. 157, 79–84.

Bassenge, E., 1995. Control of coronary blood flow by autacoids. Basic Res. Cardiol. 90, 125–141.

Brenner, B.M., Ballermann, B.J., Gunning, M.E., Zeidel, M.L., 1990. Diverse biological actions of atrial natriuretic peptide. Physiol. Rev. 70, 665–699.

Buckley, M.G., Markandu, N.D., Miller, M.A., Sagnella, G.A., MacGregor, G.A., 1993. Plasma concentrations and comparisons of brain and atrial natriuretic peptide in normal subjects and in patients with essential hypertension. J. Hum. Hypertens. 7, 245–250.

Chauhan, A., More, R.S., Mullins, P.A., Taylor, G., Petch, C., Schofield, P.M., 1996. Aging-associated endothelial dysfunction in humans is reversed by L-arginine. J. Am. Coll. Cardiol. 28, 1796–1804.

Dietz, J.R., 1987. Control of atrial natriuretic factor release from a rat heart–lung preparation. Am. J. Physiol. 252, 498–502.

Ding, J., Thibault, G., Gutkowska, J., Garcia, R., Karabatsos, T., Jasmin, G., Genest, J., Cantin, M., 1987. Cardiac and plasma atrial natriuretic factor in experimental congestive heart failure. Endocrinology 121, 248–257.

Dusting, G.J., 1996. Nitric oxide in coronary artery disease: roles in atherosclerosis, myocardial reperfusion and heart failure. EXS 76, 33–55.

Elizabeth, A.K., Seth, P., Fung, H.-L., 1992. Metabolic activation of sodium nitroprusside to nitric oxide in vascular smooth muscle. J. Pharmacol. Exp. Ther. 262, 916–922.

Engler, M.B., Engler, M.M., 1996. Influence of aging on the relaxant response to ω-3 fatty acids in Fischer 344 rat aorta. Gerontology 42, 25–35.

Granger, D.L., Taintor, R.R., Boockvar, K.S., Hibbs, J.J., 1996. Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction. Methods Enzymol. 268, 142–151.

Ignarro, L.J., Lippton, H., Edwards, J.C., Baricos, W.H., Hyman, A.L., Kadowitz, P.J., Gruetter, C.A., 1981. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: evidence for the involvement of *S*-nitrosothiols as active intermediates. J. Pharmacol. Exp. Ther. 218, 739–749.

Ishihata, A., Endoh, M., 1993. Pharmacological characteristics of the positive inotropic effect of angiotensin II in the rabbit ventricular myocardium. Br. J. Pharmacol. 108, 999–1005.

Ishihata, A., Endoh, M., 1995. Species-related differences in inotropic effects of angiotensin II in mammalian ventricular muscle: receptors, subtypes and phosphoinositide hydrolysis. Br. J. Pharmacol. 114, 447–453.

Katano, Y., Ishihata, A., Morinobu, S., Endoh, M., 1993. Modulation by aging of the coronary vascular response to endothelin-1 in the rat isolated perfused heart. Naunyn-Schmiedeberg's Arch. Pharmacol. 348, 82–87.

Küng, C.F., Lüscher, T.F., 1995. Different mechanisms of endothelial dysfunction with aging and hypertension in rat aorta. Hypertension 25, 194–200.

Lamontagne, D., König, A., Bassenge, E., Busse, R., 1992. Prostacyclin and nitric oxide contribute to the vasodilator action of acetylcholine and bradykinin in the intact rabbit coronary bed. J. Cardiovasc. Pharmacol. 20, 652–657.

Liu, Q., Nakae, I., Tsutamoto, T., Takaoka, A., Kinoshita, M., 1996. In vivo vasodilatory action of atrial natriuretic peptides in canine coronary circulation. Jpn. Circ. J. 60, 300–310.

Lyons, D., 1997. Impairment and restoration of nitric oxide-dependent vasodilation in cardiovascular disease. Int. J. Cardiol. 62 (Suppl. 2), S101–S109.

Magness, R.R., Rosenfeld, C.R., Hassan, A., Shaul, P.W., 1996. Endothelial vasodilator production by uterine and systemic arteries: I. Effects of ANG II on PGI2 and NO in pregnancy. Am. J. Physiol. 270, H1914–H1923.

Melo, L.G., Sonnenberg, H., 1996. Effect of nitric oxide inhibition on secretion of atrial natriuretic factor in isolated rat heart. Am. J. Physiol. 270, H306–H311.

Misko, T.P., Schilling, R.J., Salvemini, D., Moore, W.M., Currie, M.G., 1993. A fluorometric assay for the measurement of nitrite in biological samples. Anal. Biochem. 214, 11–16.

Mohazzab-H, K.M., Kaminski, P.M., Agarwal, R., Wolin, M.S., 1999. Potential role of a membrane-bound NADH oxidoreductase in nitric oxide release and arterial relaxation to nitroprusside. Circ. Res. 84, 220–228.

Nishikimi, T., Yoshihara, F., Morimoto, A., Ishikawa, K., Ishimitsu, T., Saito, Y., Kangawa, K., Matsuo, H., Omae, T., Matsuoka, H., 1996. Relationship between left ventricular geometry and natriuretic peptide levels in essential hypertension. Hypertension 28, 22–30.

Noll, G., Wenzel, R.R., Lüscher, T.F., 1996. Endothelin and endothelin antagonists: potential role in cardiovascular and renal disease. Mol. Cell. Biochem. 157, 259–267.

Ontkean, M., Gay, R., Greenberg, B., 1991. Diminished endothelium-derived relaxing factor activity in an experimental model of chronic heart failure. Circ. Res. 69, 1088–1096.

Oriji, G.K., Keiser, H.R., 1997. Protein kinase C mediates angiotensin II-induced contractions and the release of endothelin and prostacyclin in rat aortic rings. Prostaglandins, Leukotrienes Essent. Fatty Acids 57, 135–141.

Pörsti, I., Hecker, M., Bassenge, E., Busse, R., 1993. Dual action of angiotensin II on coronary resistance in the isolated perfused rabbit heart. Naunyn-Schmiedeberg's Arch. Pharmacol. 348, 650–658.

Sagnella, G.A., Markandu, N.D., Shore, A.C., MacGregor, G.A., 1986. Raised circulating levels of atrial natriuretic peptides in essential hypertension. Lancet 1, 179–181.

Sanchez, F.C., Burnett, J.J., Lorenz, R.R., Vanhoutte, P.M., 1990. Possible modulation of release of atrial natriuretic factor by endothelium-derived relaxing factor. Am. J. Physiol. 259, H982–H986.

Schiebinger, R.J., Gomez, S.C., 1990. Endothelin: a potent stimulus of atrial natriuretic peptide secretion by superfused rat atria and its dependency on calcium. Endocrinology 127, 119–125.

Sonnenberg, H., Veress, A.T., 1984. Cellular mechanism of release of atrial natriuretic factor. Biochem. Biophys. Res. Commun. 124, 443–449.

Tschudi, M.R., Barton, M., Bersinger, N.A., Moreau, P., Cosentino, F., Noll, G., Malinski, T., Lüscher, T.F., 1996. Effect of age on kinetics of nitric oxide release in rat aorta and pulmonary artery. J. Clin. Invest. 98, 899–905.

Volpe, M., Atlas, S.A., Sosa, R.E., Marion, D.E., Mueller, F.B., Sealey, J.E., Laragh, J.H., 1990. Angiotensin II-induced atrial natriuretic factor release in dogs is not related to hemodynamic responses. Circ. Res. 67, 774–779.

Yamazaki, M., Toda, N., 1991. Comparison of responses to angiotensin II of dog mesenteric arteries and veins. Eur. J. Pharmacol. 201, 223–229.