ELSEVIER

European Journal of Pharmacology 320 (1997) 175–181

ejp

Age-related differences and roles of endothelial nitric oxide and prostanoids in angiotensin II responses of isolated, perfused mesenteric arteries and veins of rats

Chika Konishi [a], Yukiko Naito [a], Yoshiaki Saito [b], Naoki Ohara [a,*], Hiroshi Ono [a]

[a] *Department of Pharmacology, Hatano Research Institute, Food and Drug Safety Center, Ochiai 729-5, Hadano, Kanagawa 257, Japan*
[b] *Department of Pathology, Hatano Research Institute, Food and Drug Safety Center, Ochiai 729-5, Hadano, Kanagawa 257, Japan*

Received 11 July 1996; revised 12 November 1996; accepted 15 November 1996

Abstract

We examined whether or not cyclo-oxygenase products of arachidonic acid and endothelium-derived relaxing factor (nitric oxide, NO) regulate the vascular response to angiotensin II differently with aging or development. For this purpose angiotensin II responses of isolated, perfused rat mesenteric vascular beds were compared between rats aged 4 weeks and 32 weeks. Angiotensin II increased perfusion pressure in arteries and veins of both rats aged 4 weeks and 32 weeks. In the arteries of rats aged 32 weeks the increase was slight, and less than that in rats aged 4 weeks. In contrast, the veins showed similar increases in perfusion pressure in rats aged 4 weeks and 32 weeks. Indomethacin, an inhibitor of cyclo-oxygenase, at 5×10^{-6} M depressed the increase in perfusion pressure only in the arteries of rats aged 32 weeks. N^{G}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide (NO) synthase, applied at 5×10^{-6} M in the presence of indomethacin enlarged the perfusion pressure increase in the arteries of both rats aged 4 weeks and 32 weeks, while it failed to modify that in the veins. After removal of the endothelium from the blood vessels, the perfusion pressure responses in arteries were increased in both rats aged 4 weeks and 32 weeks, whereas those in veins were not affected. Regardless of the endothelium being intact or removed, the increase in arterial perfusion pressure of rats aged 32 weeks all but disappeared with 5×10^{-6} M furegrelate, an inhibitor of thromboxane A_2 synthase, and with a combined application of furegrelate and 10^{-6} M SQ29,548, a blocker of thromboxane A_2/prostaglandin H_2 receptors. These results indicate the following: in rat mesenteric vascular beds the angiotensin II response in the arteries appears to diminish with aging or development, whereas that in the veins does not change. The NO released from the endothelium regulates the arterial response but vasodilating prostanoids have no role in the response. Moreover, in the arteries of rats aged 32 weeks, vasoconstricting prostanoids, such as prostaglandin H_2 and thromboxane A_2, seem to play a role in angiotensin II-induced vasoconstriction. With aging or development, and depending on the type of blood vessel, NO and prostanoids appear to modify the angiotensin II response differently.

Keywords: Angiotensin II; EDRF (endothelium-derived relaxing factor); Prostanoid; Mesenteric artery; Mesenteric vein; Aging; Development

1. Introduction

Vascular smooth muscle contracts in response to angiotensin II through stimulation of specific receptors located on the endothelial and the smooth muscle cell membrane. The stimulus prompts inositol-1,4,5-triphosphate (IP_3) formation with a subsequent increase in cytosolic calcium concentration and diacylglycerol formation, which leads to the activation of protein kinase C (Timmermans et al., 1993). Angiotensin II also stimulates the release of arachidonic acid metabolites from both endothelial and smooth muscle cells (Nwator et al., 1989; Timmermans et al., 1993; Catalioto et al., 1996). Of these metabolites, prostaglandin E_2 and prostaglandin I_2 have been reported to regulate angiotensin II-induced contraction (Nwator et al., 1989; Toda et al., 1990; Encabo et al., 1994), though the effects appeared to vary among the different types of blood vessel and among species (Minami and Toda, 1988; Toda et al., 1990; Yamazaki and Toda, 1991; Yoshida et al., 1991; Leung et al., 1992). Also nitric oxide (NO) released from the endothelium may modify the response to the peptide (Gruetter et al., 1988; Zhang et al., 1994, 1995). However, it has been reported that angiotensin II

* Corresponding author. Tel.: (81-463) 82-4751; Fax: (81-463) 82-9627.

PII S0014-2999(96)00913-2

stimulates the production of contractile prostanoids, such as thromboxane A_2 and prostaglandin H_2 (Toda et al., 1990; Kaushal and Wilson, 1990; Wilcox et al., 1991). Additionally, the participation of lipoxygenase metabolites of arachidonic acid in the response has also been suggested (Stern et al., 1989).

Age-related changes in the vascular responses to vasoactive substances have been extensively studied and reviewed, especially with respect to the responses to bioactive amines or to acetylcholine (Vanhoutte, 1988; Docherty, 1990; Lakatta, 1993). Also, several studies have been carried out to investigate the age-related changes in the vascular responses to angiotensin II. In rat aorta, an age-related decrease in angiotensin II response has been reported (Schoepp and Rutledge, 1984; Wakabayashi et al., 1990). In contrast, in dog coronary and mesenteric arteries, no age-related changes have been reported (Toda et al., 1987; Toda and Shimizu, 1987). Additionally, receptor binding studies have demonstrated no age-related changes in angiotensin II receptor density in rat glomeruli (Chatziantoniou and Arendshorst, 1991), but an age-related decrease in angiotensin AT_1 receptor density in rat aorta (Viswanathan et al., 1991). The release of NO from the endothelium, which seems to regulate the angiotensin II response in the blood vessels (Gruetter et al., 1988; Zhang et al., 1994, 1995), has been reported to decrease age-relatedly in the endothelium of rat aorta (Moritoki et al., 1992) and of human umbilical veins (Sato et al., 1993).

Given this background, it is of interest to investigate the changes in angiotensin II responses with aging or development in relation to differences in the roles of arachidonic acid metabolites and NO in the response. Moreover, most of the above-mentioned studies did not use vascular beds, but rather the relatively large conduit arteries, and there are no reports in which both arterial and venous responses were examined simultaneously. In the present study, age-related, especially developmental, changes in the features of the vascular response to angiotensin II were examined using isolated, perfused rat mesenteric arteries and veins.

Part of the present study has been presented at the 93rd Kanto District Meeting of Japanese Pharmacological Society in Hamamatsu, October 7th, 1995.

2. Materials and methods

2.1. Isolated, perfused mesenteric vascular beds

Seventy male Wistar rats of 4 weeks, weighing 82.4–145.0 g, and of 32 weeks, weighing 508.0–666.2 g were used following the instructions of the Committee for Ethical Usage of Experimental Animals in Hatano Research Institute. The animals were anesthetized with intraperitoneal injection of sodium pentobarbital at 50 mg/kg, and were intravenously given sodium heparin at 100 U/kg. Subsequently they were killed by exsanguination, and an abdominal incision was made. Then, an isolated perfused preparation of rat mesenteric vascular bed was made in accordance with Warner's method (Warner, 1990). Briefly, polyethylene cannulae were inserted into the superior mesenteric artery with the ileocolic and colic branches being ligated, and retrogradely into the portal vein. Modified Krebs-Henseleit solution (the composition (mM) was as follows: NaCl, 118; KCl, 4.7; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25 and glucose, 11, containing aminobenzylpenicillin sodium at 50 IU/ml to avoid decomposition of the preparation), being warmed to 38°C and aerated with 95% O_2-5% CO_2 gas, was sent through a circuit of vinyl tubes to the arterial cannula at a constant rate of 3–3.5 ml/min, by means of a peristaltic pump (MP-3, Tokyo Rikakikai). The physiological saline was drained through the cannula placed in the portal vein. After a 5-min rinse by perfusion, the intestine was removed by cutting the mesentery closely along the digestive tract. The cannula at the portal vein was then also connected to the circuit, and the venous portion of the vascular bed was perfused retrogradely. After a 45-min equilibration, angiotensin II was injected using a microsyringe into rubber tubes which were placed in the circuit at positions close to each cannula. The vascular responses were monitored as changes in perfusion pressure. Perfusion pressure was measured by a pressure transducer (P23XL, Spectramed) which was placed in the circuit between the outlet of the pump and the preparation for each arterial or venous portion.

It is well known that repeated application of angiotensin II gives rise to a diminution of the response because of tachyphylaxis (Toda et al., 1990; Oshiro et al., 1989; Ullian and Linas, 1990; Frediani-Neto et al., 1991; Pörsti et al., 1993). To avoid such a reduction in the response during a series of experiments, firstly we repeatedly (3 times) injected 0.1 nmol angiotensin II, which caused a sub-maximal response, with 45-min resting intervals in order to obtain reproducible responses. Thereafter the control response was recorded.

2.2. Angiotensin II responses in preparations from rats aged 4 weeks and 32 weeks with and without endothelium

Angiotensin II (0.01–0.3 nmol) was injected and dose-response relationships in the arteries and in the veins with endothelium were compared between rats aged 4 weeks and 32 weeks.

In another series of experiments the control responses to angiotensin II at 0.1 nmol were examined in rats aged 4 weeks and 32 weeks. Then the endothelium was chemically removed by injecting 1 ml of 1% 3-[(3 cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) solution. Endotherium denudation was confirmed by the abolition of acetylcholine-induced dilation in arteries preconstricted by phenylephrine at 10^{-5} M, and of bradykinin-induced dilation in veins preconstricted by U-

46619 at 10^{-7} M. Thereafter the angiotensin II responses were examined again in rats aged 4 weeks and 32 weeks. Afterwards the vascular beds were examined for endothelium denudation under an electron microscope.

In several preparations with and without endothelium, [Sar[1],Ile[8]]angiotensin II, a blocker of the angiotensin II receptor, was used at 10^{-8} M to confirm whether or not angiotensin II responses were produced through the stimulation of specific angiotensin II receptors.

2.3. Modification by prostanoids and nitric oxide (NO) of angiotensin II response

After the control angiotensin II (0.1 nmol) responses in the preparations with endothelium were recorded, indomethacin at 5×10^{-6} M was added to physiological saline. The responses were observed after a 30-min equilibration period. Additionally, N^G-nitro-L-arginine methyl ester (L-NAME) at 5×10^{-6} M was added to physiological saline containing indomethacin, and changes in angiotensin II response were examined again. The concentration of L-NAME was enough to inhibit the production of NO, since in this preparation L-NAME at 10^{-4} M caused no additional increase in angiotensin II responses.

In some arterial preparations from rats of 32 weeks, the effect of a thromboxane A_2 synthase inhibitor, furegrelate (Kaushal and Wilson, 1990), on the angiotensin II response was examined in preparations with and without endothelium at 5×10^{-6} M in physiological saline. Thereafter SQ 29,548 at 10^{-6} M was added to physiological saline in combination with furegrelate to confirm whether the blockade of thromboxane A_2/prostaglandin H_2 receptors caused further changes in the angiotensin II response.

2.4. KCl-induced contracture

Physiological saline containing 100 mM KCl was prepared by replacing a sufficient amount of sodium chloride in physiological saline with potassium chloride. The arterial and venous preparations with endothelium were perfused with this high K^+ physiological saline and the full contractures were compared between rats aged 4 weeks and 32 weeks. Additionally, these responses were also recorded in the presence of L-NAME at 5×10^{-6} M to examine the modification by endothelial NO of the contractures.

2.5. Chemicals

The following chemicals were used in the present study: acetylcholine chloride (Wako, Osaka, Japan); aminobenzyl penicillin sodium (Asahi, Osaka, Japan); angiotensin II (human), [Sar[1],Ile[8]]angiotensin II and bradykinin (Peptide Institute, Osaka, Japan); (3-[(3 cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS, Bio-Rad, Tokyo, Japan), furegrelate, N^G-nitro-L-arginine methyl ester (L-NAME) and 7-[3-[[2-[(phenylamino)carbonyl]hydrazino]-methyl]-7-oxabicyclo[2,2,1]hept-2-yl]-, [1*S*(1α,2α,(*Z*), 3α,4α)]-5-heptenoic acid (SQ 29,548) (Funakoshi, Tokyo, Japan); indomethacin and phenylephrine hydrochloride (Sigma-Aldrich Japan, Tokyo, Japan); and 9,11-dideoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U-46619, Cayman, Ann Arbor, MI, USA). All other chemicals were of the purest grade commercially available.

2.6. Statistics

The results in the present study are presented as means with standard errors. The difference in group means was evaluated by anarysis of variance (ANOVA) followed by paired *t*-test, unpaired *t*-test or Fisher's protected least significant difference (PSLD) test.

3. Results

3.1. Angiotensin II responses in arteries and veins of rats aged 4 weeks and 32 weeks

Repeated injection of angiotensin II reduced the vascular response. Namely, in each preparation, the initial increase in perfusion pressure was approximately 2–3 times as large as the responses after repeated injections. In these preparations the veins from both rats aged 4 weeks and 32 weeks showed comparable increases in perfusion pressure, and the maximum perfusion pressure was 5.1 ± 1.6 mmHg in rats of 4 weeks and 6.2 ± 1.8 mmHg in rats of 32 weeks. The maximum perfusion pressure in the arteries was lower for rats of 32 weeks, 1.4 ± 0.5 mmHg, than for rats of 4 weeks, 7.5 ± 2.4 mmHg (Fig. 1).

After removal of the endothelium by injection of 1% CHAPS, acetylcholine-induced dilation in the arteries and bradykinin-induced dilation in the veins were abolished in preparations from both rats aged 4 weeks and 32 weeks (data not shown). We also confirmed the denudation under an electron microscope. The denudation potentiated angiotensin II (0.1 nmol) responses in the arteries of both rats of 4 weeks, from 7.5 ± 2.4 mmHg to 28.5 ± 4.3 mmHg, and rats of 32 weeks, from 1.4 ± 0.5 mmHg to 9.2 ± 2.7 mmHg, whereas there were no significant changes in the venous responses (Fig. 2). The arterial and venous angiotensin II responses were completely inhibited by [Sar[1],Ile[8]]angiotensin II, whether the endothelium was intact or removed. The control angiotensin II responses in vessels from rats of 32 weeks (arterial perfusion pressure/venous perfusion pressure in mmHg, $n = 3$) were $1.6 \pm 0.8/7.1 \pm 1.5$ and $10.0 \pm 3.5/5.4 \pm 1.3$, in the preparations with and without the endothelium, respectively. In vessels from rats of 4 weeks the responses (arterial perfusion pressure/venous perfusion pressure in mmHg, $n = 3$) were $11.5 \pm 5.0/5.5 \pm 0.9$ and $29.7 \pm$

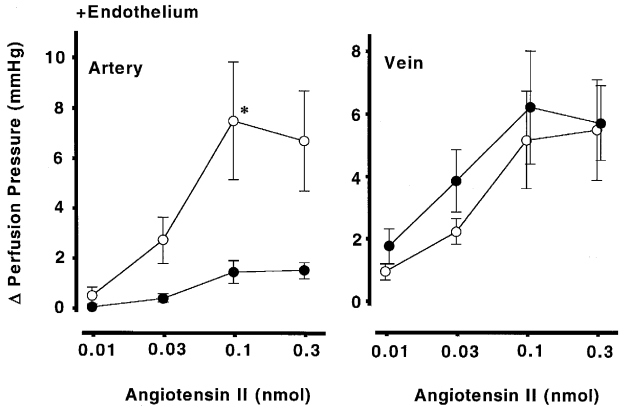

Fig. 1. Dose–response curves for angiotensin II in isolated perfused mesenteric vascular beds with endothelium in rats of different ages. Symbols with vertical bars represent mean values with standard errors of 6–10 experiments. Open and filled circles represent the responses in vascular beds of 4-week-old and 32-week-old animals, respectively. * $P < 0.05$, significantly different from the response of 32-week-old rats.

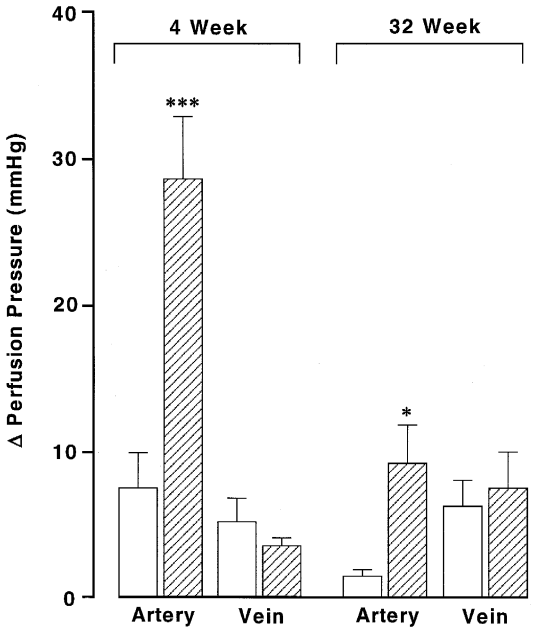

Fig. 2. The influence of endothelium denudation on angiotensin II responses of isolated perfused mesenteric vascular beds in rats of different ages. The changes in perfusion pressure produced by 0.1 nmol of angiotensin II are shown. Columns with vertical bars represent mean values of the increase in the perfusion pressure with standard errors for 7–12 experiments. Open columns indicate the responses in the preparations with endothelium, and hatched columns indicate that without endothelium. * $P < 0.05$, *** $P < 0.001$ significantly different from the response in the preparations with endothelium.

3.1/4.2 ± 0.5, in the preparations with and without the endothelium, respectively. All these responses disappeared (perfusion pressure reduced to 0 mmHg) after addition of [Sar[1],Ile[8]]angiotensin II.

3.2. Participation of prostanoids and NO

The perfusion pressure increase elicited by angiotensin II in the arteries and veins from rats aged 4 weeks, and that in the veins from rats aged 32 weeks, were not changed by indomethacin, while the response of arteries from rats aged 32 weeks was diminished to 19.7 ± 12.1% of the control response (Fig. 3). After addition of L-NAME in the presence of indomethacin, the angiotensin II responses were potentiated to 221.8 ± 36.4% and to 166.5 ± 41.2% of the control in arteries from rats aged 4 weeks and 32 weeks, respectively. However, the venous responses were not much modified (Fig. 3).

In rats aged 32 weeks, the angiotensin II response of the arteries with endothelium was abolished by furegrelate. Supplementary SQ 29,548 in the presence of furegrelate no longer changed the response. In the preparations without endothelium, however, furegrelate markedly inhibited the response but it was not abolished. A combined treatment with SQ 29,548 and furegrelate tended to decrease the response further (Fig. 4).

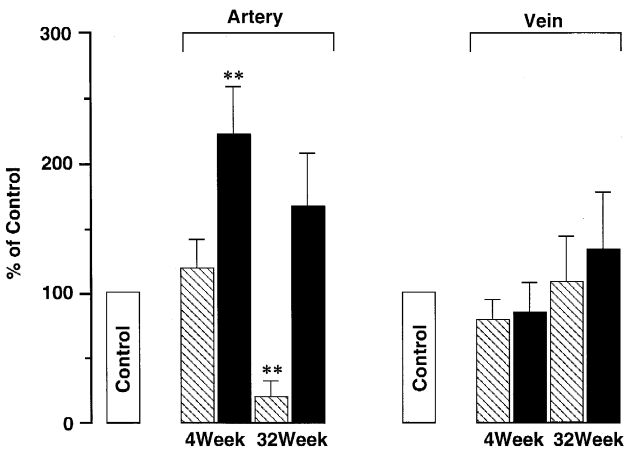

Fig. 3. Effects of indomethacin and L-NAME on angiotensin II responses of isolated mesenteric vascular beds with endothelium in rats of different ages. The changes in perfusion pressure produced by 0.1 nmol of angiotensin II are shown. After the control responses were recorded, the effects of indomethacin and of the combination of indomethacin and L-NAME on the responses were succesively examined. Columns with vertical bars represent responses as a percentage of control (in the absence of indomethacin and L-NAME), means with standard errors of 7–10 experiments. Hatched columns represent the responses in the presence of 5×10^{-6} M indomethacin. Filled columns represent the responses in the presence of 5×10^{-6} M L-NAME with indomethacin. All the responses are for preparations with the endothelium. ** $P < 0.01$, significantly different from the control response (100%).

3.3. KCl-induced contracture

There was no difference between rats aged 4 weeks and 32 weeks in the sustained increases in the arterial perfusion pressure produced by 100 mM KCl. Further increases in the perfusion pressure following addition of L-NAME to the high K^+ physiological saline were similar in rats aged 4 weeks and 32 weeks (Fig. 5). The contraction produced by a high concentration of K^+ was also observed in veins.

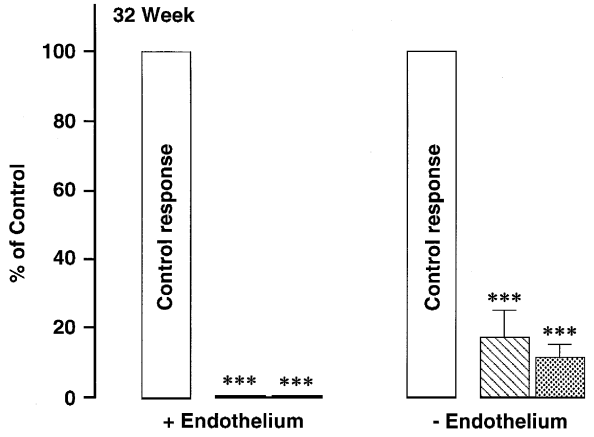

Fig. 4. Effects of furegrelate and SQ 29,548 on angiotensin II responses of isolated perfused mesenteric arteries from 32-week-old rats. The changes in perfusion pressure produced by 0.1 nmol of angiotensin II are shown. After the control responses were recorded, the effects of furegrelate and of the combination of furegrelate and SQ 29,548 on the responses were succesively examined. Columns with vertical bars represent responses as a percentage of the control, means and standard errors of 8 experiments. Hatched columns demonstrate the responses in the presence of 5×10^{-6} M furegrelate and shaded columns the response in the presences of 10^{-6} M SQ 29,548 with furegrelate. + and − Endothelium indicate the results for preparations with and without endothelium, respectively. *** $P < 0.001$, significantly different from the control response (100%).

However, the magnitude of the response was much smaller than that in the arteries. L-NAME potentiation of the venous response was not clear (data not shown).

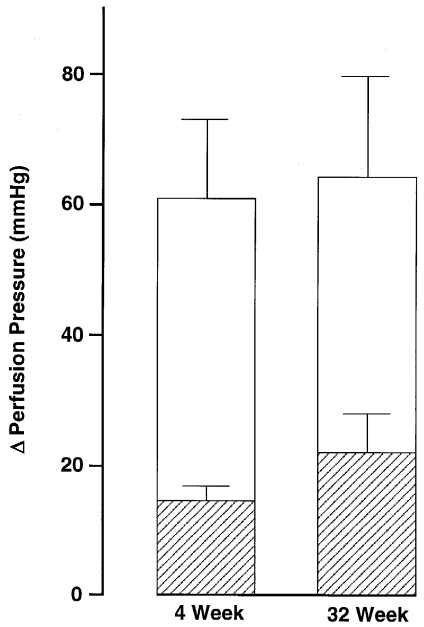

Fig. 5. Potentiation of KCl-induced contracture of isolated perfused mesenteric arteries with endothelium in rats of different age. Increases in perfusion pressure by perfusing with physiological saline containing 100 mM KCl (hatched parts) and additional increases in perfusion pressure after addition of 5×10^{-6} M L-NAME in physiological saline. Columns with vertical bars represent mean values with standard errors of 10 experiments.

4. Discussion

In the mesenteric vascular beds of adult rats, Warner (1990) has reported that angiotensin II causes a contraction not in arteries, but in veins. This was confirmed in the present study. That is, in rats of 32 weeks almost no changes in the perfusion pressure of arteries were found after angiotensin II injection, while in rats aged 4 weeks, the arteries responded well to the peptide. In contrast with the perfusion pressure of arteries, the perfusion pressure of veins from both rats aged 4 weeks and 32 weeks was similarly increased by angiotensin II injection. These findings indicate that in the mesenteric arteries of mature rats, angiotensin II does not act as a noticeable vasoconstrictor, and that the response to angiotensin II decreases with aging or development. Actually, we observed that the angiotensin II response was abolished in the arteries of 70-week-old rats (data not shown).

The endothelium was chemically disrupted to examine its role in the diverse responses to angiotensin II of the vascular beds. Endothelium denudation potentiated only the arterial responses, and the increase in the perfusion pressure of arteries from rats of 4 weeks was still larger than that in arteries from rats of 32 weeks. Thus it is suggested that the endothelium negatively regulates the arterial contraction to angiotensin II, though this regulation is not particularly stronger in rats of 32 weeks than in rats of 4 weeks, and that the venous angiotensin II response is not modified by the endothelium. In addition, to confirm the role of endothelial NO in angiotensin II responses, L-NAME was added to physiological saline, and angiotensin II response of the arteries with an intact endothelium was examined. After addition of L-NAME to physiological saline, the response was enlarged only in the arteries, and the change was not specifically pronounced in rats of 32 weeks in comparison with rats of 4 weeks. Moreover, the regulatory ability of endothelial NO on the mechanical response was compared between the preparations from rats aged 4 weeks and 32 weeks which were contracted by 100 mM KCl. In the presence of L-NAME, the KCl-induced increase in arterial perfusion pressure was further increased, and the magnitude of the additional increase was similar in rats aged 4 weeks and 32 weeks. These results demonstrate that the regulation by the endothelium of the angiotensin II response is found only in arteries, and that NO or NO-related substances derived from the endothelium play a role in this regulation.

Angiotensin II has been reported to release vasodilating prostanoids, such as prostaglandin E_2, and prostaglandin I_2, from both the endothelium and the smooth muscle (Toda et al., 1990; Nwator et al., 1989; Encabo et al., 1994). Thus, in order to examine whether these prostanoids also regulate angiotensin II responses differently between rats aged 4 weeks and 32 weeks, the responses were compared in the presence or absence of indomethacin, using preparations with an intact endothelium. However,

neither the arterial response nor the venous one was potentiated by the cyclo-oxygenase inhibitor in preparations from either rats of 4 weeks or 32 weeks. Therefore, the vasodilating prostanoids appear not to have a role in modifying the response.

So far, NO derived from the endothelium and the vasodilating prostanoids can be ruled out as factors producing the difference in the arterial angiotensin II response between rats aged 4 weeks and 32 weeks. Despite the age-associated difference, the angiotensin II responses were mediated through specific receptors, since the responses in both preparations, with and without the endothelium, were completely inhibited by [Sar^1,Ile^8]angiotensin II. Moreover, between rats aged 4 weeks and 32 weeks, there were no significant age-related alterations in 100 mM KCl-induced perfusion pressure increases in the arteries, indicating that the contracting potencies in preparations from rats aged 4 weeks and 32 weeks were comparable. Therefore, it is possible that the age-related change in arterial angiotensin II response may be due to a difference in the number of specific receptors or to the post-receptor mechanisms in the smooth muscle cells which link the stimuli of the receptors to the contractile protein.

It has been reported that in rat aorta and mesenteric arteries the dilation response to acetylcholine or ATP decreases with age (Hongo et al., 1988; Mantelli et al., 1995). We also found an age-related decrease in acetylcholine- or ATP-induced dilation in the perfused mesenteric arteries of rats (data not shown). Thus it is conceivable that the release of endothelial NO through the stimuli of NO-releasing vasodilators decreases with age. However, in the present study, the L-NAME potentiation of the angiotensin II response (indicating regulatory control by NO) was not necessarily diminished in proportion to the control response in rats of 32 weeks compared with that in rats of 4 weeks. Additionally, the similar KCl-induced increases in arterial perfusion pressure in preparations from rats aged 4 weeks and 32 weeks were further increased by L-NAME by the same magnitude. Therefore, it is likely that the release of NO triggered by vasoconstricting agents for regulation of the responses has different characteristics from that triggered by vasodilating agonists.

Interestingly, indomethacin blocked angiotensin II response in the arteries of rats of 32 weeks. The angiotensin II response in the arteries with an intact endothelium was so small that it might not be important as a local vascular response. However, the mesenteric vascular bed is one of the important parts of the circulatory system in controlling systemic blood distribution and blood pressure. Therefore even such a small change may have an important role in the function of the circulation throughout life. Thus, we analyzed the angiotensin II response using pharmacological tools. The response was inhibited by indomethacin, furegrelate and the combined treatment with furegrelate and SQ 29,548, demonstrating that contractile prostaglandins, such as prostaglandin H_2 and thromboxane A_2, play a role in the angiotensin II contraction. The angiotensin II response was inhibited by furegrelate in the arteries with endothelium. A strong inhibition of the response by furegrelate and further inhibition by the combined treatment with furegrelate and SQ 29,548 were observed in the arteries without endothelium. Therefore, thromboxane A_2 synthesis seems to play a key role in the angiotensin II response. An additional interesting finding was that furegrelate and the combined treatment with furegrelate and SQ 29,548 inhibited the angiotensin II responses in both preparations, with and without endothelium, but the inhibitory effect was pronounced in the preparation with endothelium. Actually, it has been reported that the major origin of the prostanoids in blood vessels may be the endothelium (Lin and Nasjletti, 1991), though smooth muscle cells are also known to release them (Shirahase et al., 1987). The present finding may reflect such a difference in potency to release vasoconstricting prostanoids through angiotensin II stimulation. In the presence of the endothelium, angiotensin II might contract the arteries indirectly, by releasing prostanoids from the endothelium. In contrast, in the absence of the endothelium, the peptide might contract the arteries predominantly through a direct action on specific receptors.

In conclusion, with aging or development, the angiotensin II response in the venous portion of the rat mesenteric vascular bed does not change, but the arterial response diminishes. The arterial response is regulated by endothelial NO, whereas the venous response is not. Cyclo-oxygenase products, such as prostaglandin H_2 and thromboxane A_2, seem to play a role in angiotensin II-induced contraction of the arteries in rats of 32 weeks, though the magnitude of the response is small. NO derived from the endothelium and prostanoids modify the angiotensin II-induced responses of arteries and veins differently with age, though they do not appear to be major mechanisms underlying the age-related decreases in angiotensin II responses in arteries.

Acknowledgements

The authors would like to thank Mr. D.L. Carleton for his excellent help in the preparation of this manuscript. This work was supported by the HEART 21 promotion fund from the Ministry of Health and Welfare, Japan. A part of this study has been presented at the 93rd Kanto District Meeting of the Japanese Pharmacological Society in Hamamatsu, October 7, 1995.

References

Catalioto, R.-M., A.R. Renzetti, M. Criscuoli, L. Morbidelli and A. Subissi, 1996, Role of calcium in angiotensin II-induced prostaglandin release and DNA synthesis in rat vascular smooth muscle cells, J. Cardiovasc. Pharmacol. 27, 195.

Chatziantoniou, C. and W.J. Arendshorst, 1991, Angiotensin and thromboxane in genetically hypertensive rats: renal blood flow and receptor studies, Am. J. Physiol. 261, F238.

Docherty, J.R., 1990, Cardiovascular responses in ageing: a review, Pharmacol. Rev. 42, 103.

Encabo, A., M. Ferrer, J. Marín and G. Balfagón, 1994, Angiotensin modulation of vascular tone and adrenergic neurotransmission in cat femoral arteries, Gen. Pharmacol. 25, 1691.

Frediani-Neto, E., E.G. Silva, T.B. Paiva and A.C.M. Paiva, 1991, Increased intracellular Na^+ and depolarization favor angiotensin tachyphylaxis in rabbit aorta, Am. J. Physiol. 260, H373.

Gruetter, C.A., E.T. Ryan, S.M. Lemke, D.A. Bailly and M.K. Fox, 1988, Endothelium-dependent modulation of angiotensin II-induced contraction in blood vessels, Eur. J. Pharmacol. 146, 85.

Hongo, K., T. Nakagomi, N.F. Kassell, T. Sasaki, M. Lehman, D.G. Vollmer, T. Tsukahara, H. Ogawa and J. Torner, 1988, Effects of aging and hypertension on endothelium-dependent vascular relaxation in rat carotid artery, Stroke 19, 892.

Kaushal, R.D. and T.W. Wilson, 1990, Effect of furegrelate on renal plasma flow after angiotensin II infusion, Can. J. Physiol. Pharmacol. 68, 500.

Lakatta, E.G., 1993, Cardiovascular regulatory mechanisms in advanced age, Physiol. Rev. 73, 413.

Leung, K.H., R.S.L. Chang, V.J. Lotti, W.A. Roscoe, R.D. Smith, P.B.M.W.M. Timmermans and A.T. Chiu, 1992, AT_1 receptors mediate the release of prostaglandins in porcine smooth muscle cells and rat astrocytes, Am. J. Hypertens. 5, 648.

Lin, L. and A. Nasjletti, 1991, Role of endothelium-derived prostanoid in angiotensin-induced vasoconstriction, Hypertension 18, 158.

Mantelli, L., S. Amerini and F. Ledda, 1995, Roles of nitric oxide and endothelium-derived hyperpolarizing factor in vasorelaxant effect of acetylcholine as influenced by aging and hypertension, J. Cardiovasc. Pharmacol. 25, 595.

Minami, Y. and N. Toda, 1988, Comparison of the response to angiotensin II of isolated dog coronary and mesenteric arteries of proximal and distal portions, Arch. Int. Pharmacodyn. Ther. 293, 186.

Moritoki, H., T. Yoshikawa, T. Hisayama and S. Takeuchi, 1992, Possible mechanisms of age-associated reduction of vascular relaxation caused by atrial natriuretic peptide, Eur. J. Pharmacol. 210, 61.

Nwator, I.A.A., A.A. Parsons and E.T. Whalley, 1989, Prostanoid involvement in the relaxation of in vitro mesenteric artery by bradykinin and des-Arg^9-bradykinin, Eur. J. Pharmacol. 170, 29.

Oshiro, M.E.M., S.I. Shimuta, T.B. Paiva and A.C.M. Paiva, 1989, Evidence for a regulatory site in the angiotensin II-receptor of smooth muscle, Eur. J. Pharmacol. 166, 411.

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

Sato, I., I. Morita, K. Kaji, M. Ikeda, M. Nagao and S. Murota, 1993, Reduction of nitric oxide producing activity associated with in vitro aging in cultured human umbilical vein endothelial cell, Biochem. Biophys. Res. Commun. 195, 1070.

Schoepp, D.D. and C.O. Rutledge, 1984, Relationship between developmental changes in angiotensin II-induced contractions and stimulation of phosphatidylinositol labeling of rat aorta, J. Pharmacol. Exp. Ther. 229, 880.

Shirahase, H., H. Usui, K. Kurahashi, M. Fujiwara and K. Fukui, 1987, Possible role of endothelial thromboxane A_2 in the resting tone and contractile responses to acetylcholine and arachidonic acid in canine cerebral arteries, J. Cardiovasc. Pharmacol. 10, 517.

Stern, N., M. Golub, K. Nozawa, M. Berger, E. Knoll, N. Yanagawa, R. Natarajan, J.L. Nadler and M.L. Tuck, 1989, Selective inhibition of angiotensin II-mediated vasoconstriction by lipoxygenase blockade, Am. J. Physiol. 257, H434.

Timmermans, P.B.M.W.M., P.C. Wong, A.T. Chiu, W.F. Herblin, P. Benfield, D.J. Carini, R.J. Lee, R.R. Wexler, J.A.M. Saye and R.D. Smith, 1993, Angiotensin II receptors and angiotensin II receptor antagonists, Pharmacol. Rev. 45, 205.

Toda, N. and I. Shimizu, 1987, Neuroeffector function in mesenteric arteries isolated from beagles of different ages, J. Pharmacol. Exp. Ther. 240, 223.

Toda, N., K. Bian and S. Inoue, 1987, Age-related changes in the response to vasoconstrictor and dilator agents in isolated beagle coronary arteries, Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 359.

Toda, N., K. Ayaziki and T. Okamura, 1990, Modifications by endogenous prostaglandins of angiotensin II-induced contractions in dog and monkey cerebral and mesenteric arteries, J. Pharmacol. Exp. Ther. 252, 374.

Ullian, M.E. and S.L. Linas, 1990, Angiotensin II surface receptor coupling to inositol triphosphate formation in vascular smooth muscle cells, J. Biol. Chem. 265, 195.

Vanhoutte, P.M., 1988, Aging and vascular responsiveness, J. Cardiovasc. Pharmacol. 12 (Suppl. 8), S11.

Viswanathan, M., K. Tsutsumi, F.M.A. Correa and J.M. Saavedra, 1991, Changes in expression of angiotensin receptor subtypes in the rat aorta during development, Biochem. Biophys. Res. Commun. 179, 1361.

Wakabayashi, I., K. Sakamoto, K. Hatake, S. Yoshimoto and M. Kurahashi, 1990, Effect of age on contractile response to angiotensin II in rat aorta, Life Sci. 47, 771.

Warner, T.D., 1990, Simultaneous perfusion of rat isolated superior mesenteric arterial and venous beds: comparison of their vasoconstrictor and vasodilator response to agonists, Br. J. Pharmacol. 99, 427.

Wilcox, C.S., W.J. Welch and H. Snellen, 1991, Thromboxane mediates renal hemodynamic response to infused angiotensin II, Kidney Int. 40, 1090.

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

Yoshida, K., M. Yamazaki and N. Toda, 1991, Different modulation by cyclooxygenase inhibitors of the response to angiotensin II in monkey arteries and veins, Jpn. J. Pharmacol. 55, 469.

Zhang, J., J.C.A. van Meel, M. Pfaffendorf and J. Zhang, 1994, Endothelium-dependent, nitric oxide-mediated inhibition of angiotensin II-induced contractions in rabbit aorta, Eur. J. Pharmacol. 262, 247.

Zhang, J., M. Pfaffendorf, J.S. Zhang and P.A. van Zwieten, 1995, Influence of the vascular endothelium on angiotensin II-induced contractions in rabbit renal artery, Fundam. Clin. Pharmacol. 9, 25.