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Role of prostaglandins in urotensin II-induced vasodilatation in the coronary arteries of aged rats

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Abstract

Endothelial function is modulated by aging. The objective of this study was to elucidate whether aging influences urotensin II-induced coronary vasodilatation, and whether aging influences the production of endothelial factors in response to urotensin II. We examined the effects of urotensin II on coronary flow in Langendorff-perfused rat hearts. The production of nitric oxide (NO), prostacyclin and prostaglandin (PG)E_2 were determined in the coronary effluent of both young and aged rats. Urotensin II increased coronary flow in Langendorff-perfused hearts in both young and aged rats and vasodilation did not differ between young and aged rats. Pretreatment with a NO synthase inhibitor, N^G-nitro-L-arginine (L-NNA), significantly inhibited urotensin II-induced vasodilatation in young rats, but not in aged rats. In addition, urotensin II increased the production of NO only in young rats. On the other hand, the cyclooxygenase inhibitor diclofenac significantly attenuated the urotensin II-induced coronary vasodilatation in both young and aged rats. Urotensin II markedly increased the release of the vasodilating prostacyclin and PGE_2 into the coronary effluent. Production of these prostanoids was maintained even in the aged coronary arteries. These results indicate that the production of NO in the endothelium of coronary arteries is impaired in aged rats, and that prostacyclin and PGE_2 may play an important role in regulating urotensin II-induced coronary vasodilatation.

Keywords: Aging; Vasoactive agent; Coronary circulation; Endothelial function; Nitric oxide; Prostaglandin

1. Introduction

Urotensin II is a cyclic peptide (goby U-II) originally isolated from the *Gillichthys mirabilis* (a marine goby) urophysis neurosecretory system (Pearson et al., 1980). Recently, isopeptides of urotensin II have been cloned and found to be present in various mammalian (monkey, human, rat, mouse, and pig) species (Coulouarn et al., 1998, 1999; Ames et al., 1999; Liu et al., 1999; Mori et al., 1999). Physiologically, urotensin II has been shown to be one of the most potent mammalian vasoconstrictors (Ames et al., 1999). Although the deduced peptide sequence is slightly different in rats and humans (14 amino acid residues in rat vs. 11 amino acid residues in human), the biologically active cyclic heptapeptide region of urotensin II (Cys5–Cys10) is fully conserved among species (Coulouarn et al., 1998).

Urotensin II is mainly expressed in the neurosecretory system in fish, but is present in the cardiovascular system and motorneurons in mammals (Coulouarn et al., 1998). The specific receptor for urotensin II has been identified to be the orphan receptor GPR14 (Ames et al., 1999). It is distributed in the mammalian cardiovascular system (Maguire et al., 2000), which suggests that urotensin II may be more than a simple neuropeptide, but is also an important substance involved in the regulation of cardiovascular function. The urotensin II receptor has 7 transmembrane domains and is a member of the G-protein-coupled receptor family. Urotensin II exerts its effects via several signaling pathways that include Ca^{2+} mobilization (Ames et al., 1999). Previous studies have shown that urotensin II mediates a strong vasoconstriction in large coronary, pulmonary and carotid arteries of monkeys (Ames et al., 1999; Douglas, 2003); however, recent studies have demonstrated that urotensin II also causes vasodilatation in some small arteries of both rats and humans (Bottril et al., 2000; Stirrat et al., 2001; Zhang et al., 2003). It appears that the vascular effects of urotensin II are

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species specific and depend on endothelial function, vessel size and 'location of vascular beds. The endothelium plays an important role in regulating vascular tone and in maintaining cardiovascular function via production of factors that induce vasorelaxation and/or vasoconstriction. These factors include endothelium-derived nitric oxide (NO), vasodilating prostanoids (prostacyclin and prostaglandin (PG)E_2), endothelium-derived hyperpolarizing factor (EDHF), endothelin and other vasoconstrictors (Bassenge, 1995). Both prostacyclin and NO have been reported to contribute to the vasodilating effects of bradykinin in perfused rabbit hearts (Lamontagne et al., 1992). In addition, they are minimally released during resting conditions, but may respond to various vasoconstrictors such as endothelin, angiotensin II and to intravascular sheer stress (Yamazaki and Toda, 1991; Katano et al., 1993; Magness et al., 1996; Ishihata et al., 1999). We have shown previously that angiotensin II stimulated the release of NO and prostacyclin from endothelial cells to protect the vessels against vascular hypercontraction and tissue ischemia (Ishihata et al., 1999). Additionally, infusion of PGE_2, PGD_2 and iloprost (an analogue of prostacyclin) in isolated Langendorff-perfused rat hearts induced marked vasodilatation, which was reflected in a reduction in coronary perfusion pressure (Bouchard et al., 1994).

We have demonstrated in Langendorff-perfused hearts that the response to urotensin II in the coronary arteries of young rats is vasodilation (Katano et al., 2000). We have suggested that endothelial vasodilating factors may play a role in this effect of urotensin II. However, as free radicals may play an important role in aging-related disorders (Yoon et al., 2002), physiological function of endothelial cells such as the production of vasoactive substances is impaired by aging.

To elucidate the influence of aging on the vascular effects of urotensin II and the production of vasoactive substances in response to urotensin II, we investigated the effects of urotensin II on coronary flow in Langendorff-perfused hearts from both young and aged rats. We also revealed the involvement of nitric oxide, prostacyclin and PGE_2 in the regulation of coronary flow.

2. Materials and methods

In this study, 2–3 months old (200–250g) and 27–30 months old (320–370g) male Fischer 344 rats were obtained from Charles River Japan (Atsugi, Japan). 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 Langendorff-perfused hearts

Each rat was anesthetized with ether and decapitated and the heart removed immediately. The isolated heart was immediately perfused according to Langendorff's method under constant pressure (75 cm H_2O) at 37±0.1 °C with modified Krebs–Henseleit 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 and ascorbic acid 0.057. The buffer solution was continuously gassed 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). Urotensin II (100 nM) was injected into the coronary artery as a bolus (≈0.1 ml) over 10 s and N^G-nitro-L-arginine (L-NNA) or diclofenac were applied by continuous infusion via the rubber tubing connected to the aortic cannula (Ishihata et al., 1999; Katano et al., 2000). In the groups receiving pretreatment with either L-NNA or diclofenac, L-NNA or diclofenac were continuously infused via a syringe pump (Harvard Apparatus 940e, Mills, MA, USA) for 10 min prior to and during the application of urotensin II. The concentrations of L-NNA and diclofenac were sufficient to inhibit the synthesis of NO and PGs, respectively (Ishihata et al., 1999; Katano et al., 2000). The changes in coronary flow were expressed as percentage of the basal flow just before the injection of drugs. As the response of coronary flow to urotensin II was desensitized and not reproducible, the response to urotensin II in the presence and absence of L-NNA or diclofenac was recorded in different hearts.

2.2. Measurement of the concentration of vasodilating prostanoids (prostacyclin and PGE_2) in the coronary effluent

The concentration of 6-keto-$PGF_{1\alpha}$, which is a stable metabolite of prostacyclin, was measured by enzyme-immunoassay (Ishihata et al., 1999). In brief, coronary effluent was collected each minute just before and after the administration of urotensin II to measure the concentration of 6-keto-$PGF_{1\alpha}$. The samples were frozen and kept at −70 °C until analysis. The concentration of 6-keto-$PGF_{1\alpha}$ in the effluent was measured by using the commercially available acetylcholinesterase-based enzyme-immunoassay kit for 6-keto-$PGF_{1\alpha}$ (Cayman Chemical, Ann Arbor, MI, USA). The concentration of PGE_2 was also measured by enzyme-immunoassay as described above (Cayman Chemical). In some experiments, Langendorff-perfused hearts were treated with diclofenac (a cylooxygenase inhibitor) to ascertain whether cyclooxygenase was sufficiently activated after stimulation with urotensin II to increase the production of PGs.

2.3. Measurement of the concentration of 8-isoprostane as a marker of oxidative stress in the coronary effluent

The coronary effluent was collected and stored at −70 °C. The concentration of 8-isoprostane (8-epi-$PGF_{2\alpha}$) in the coronary perfusate was measured by enzyme-immunoassay. Briefly, the 8-isoprostane assay was based on the competition between 8-isoprostane and a conjugate of 8-isoprostane-acetylcholinesterase (8-isoprostane tracer) for a limited number of 8-isoprostane-specific rabbit antiserum binding sites. The antibody and isoprostane (either free or tracer) complex bind to mouse monoclonal anti-rabbit IgG previously attached to the well. After washing the plate, Ellman's reagent (which contains the substrate for acetylcholinesterase) was added to the well and

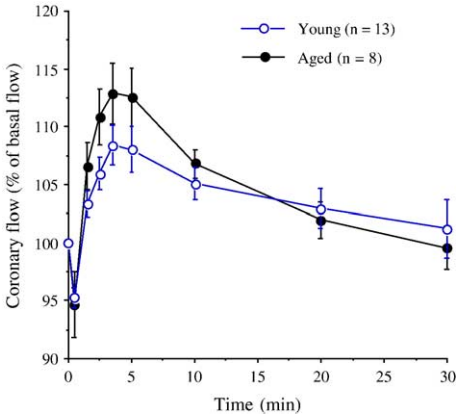

Fig. 1. Effect of urotensin II on the coronary flow in Langendorff-perfused young and aged rat hearts. Urotensin II was infused into the rubber tubing connected to the aortic cannula. Results are expressed as means±S.E.M. Responses did not differ between young and aged rats.

the absorbance of the enzymatic reaction products was measured spectrophotometrically at 405 nm (BioRad, model 550).

2.4. Measurement of the concentration of NO in the coronary effluent

Nitric oxide secreted from the coronary artery is rapidly decomposed to the 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 by Misco et al. (1993). In brief, an aliquot of coronary effluent was incubated with nitrate reductase for 1 h at 37 °C 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 acidic conditions (pH<2) at room temperature. The reaction product (naphthalenetriazole) was measured by using a spectrofluorometer (Hitachi FP-6300, Tokyo, Japan) with excitation at 365 nm and emission at 450 nm in a basic solution of 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. Materials used

Drugs used in these experiments included: acetylcholine chloride, sodium diclofenac, N^5-[nitroamidino]-L-2,5-diaminopentanoic acid (N^G-nitro-L-arginine, Sigma, St. Louis, MO, USA), sodium nitroprusside dihydrate (Wako Chemical, Osaka, Japan) and human urotensin II (Peptide Institute, Osaka, Japan). Drug solutions were freshly prepared on each experimental day.

2.6. Statistical analysis

Data were assessed by repeated measure analysis of variance (ANOVA) with multiple comparisons using Sheffe's method or unpaired *t*-test. Data are expressed as means±standard error of the mean (S.E.M). A *P*-value less than 0.05 was considered to be statistically significant.

3. Results

3.1. Effects of urotensin II on the coronary flow in young and aged rats

Fig. 1 shows the effect of urotensin II (100 nM) on coronary flow in the Langendorff-perfused heart. In the young rat, urotensin II caused a transient decrease followed by a sustained increase in coronary flow (108.4±1.7% of the basal value at 3 min, $n=13$). Thereafter, it gradually decreased and returned to the basal level within 30 min. In the aged rat, urotensin II increased the coronary flow to 112.9 ±2.7% ($n=8$) of the basal value. There was no significant difference in the urotensin II-induced vasodilatation between young and aged rats (Fig. 1).

3.2. Effects of L-NNA and diclofenac on urotensin II-induced coronary vasodilatation in young rats

In order to determine whether NO was involved in urotensin II-induced vasodilatation, we used L-NNA as an inhibitor of NO synthase. In the young rat pretreated with L-NNA (10 μM), urotensin II-induced coronary vasodilatation was significantly attenuated compared with control (Fig. 2). To determine whether PGs were involved in the urotensin II-induced vasodilation, we used diclofenac as an inhibitor of cyclooxygenase. In the young rat pretreated with diclofenac (10 μM), urotensin II-induced vasodilatation was significantly inhibited (Fig. 3). In the presence of both L-NNA and diclofenac, coronary vasodilation was completely inhibited in the young rat (Fig. 3).

3.3. Effects of L-NNA and diclofenac on the urotensin II-induced coronary vasodilatation in aged rats

Urotensin II increased coronary flow in the aged rat heart even in the presence of L-NNA (109.3±2.1% of the basal

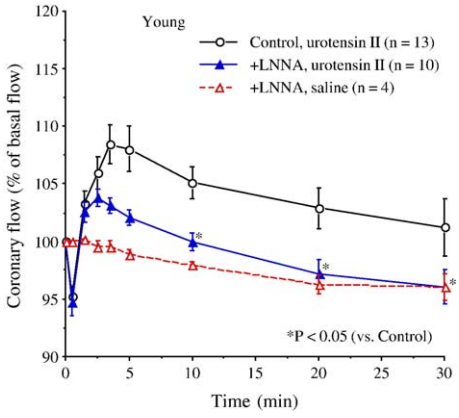

Fig. 2. Effect of nitric oxide synthase inhibitor L-NNA on urotensin II-induced coronary vasodilatation in the Langendorff-perfused young rat hearts. Continuous infusion of L-NNA (10 μM) through a syringe pump began 10 min before and continued during the application of urotensin II. Time-control for L-NNA is shown as a broken line. Results are expressed as means±S.E.M. *$P<0.05$ vs. urotensin II alone.

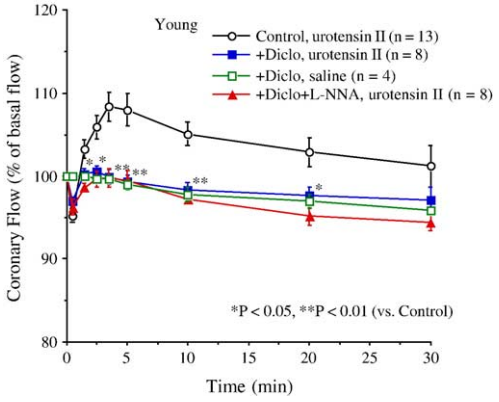

Fig. 3. Effect of cyclooxygenase inhibitor diclofenac, and combined effect of diclofenac and L-NNA on urotensin II-induced coronary vasodilatation in Langendorff-perfused young rat hearts. Continuous infusion of diclofenac (10 μM), or combination of diclofenac and L-NNA through a syringe pump started 10 min before and continued during the application of urotensin II. Results are expressed as means±S.E.M. *$P<0.05$, **$P<0.01$ vs. urotensin II alone.

value, $n=6$) and attenuation was insignificant when compared to the control group (Fig. 4). The fact that the urotensin II-induced coronary vasodilatation was significantly diminished by L-NNA only in the young rat (Fig. 3) suggests that the release of NO is stimulated by urotensin II and is involved in coronary vasodilation in young rats, but not in aged rats. On the other hand, when the aged rat heart was pretreated with diclofenac (10 μM), urotensin II-induced vasodilation disappeared (Fig. 4); this suggests involvement of cyclooxygenase products.

3.4. Effects of urotensin II on the release of prostacyclin and PGE_2 in the coronary effluent

To elucidate whether urotensin II actually stimulated the release of vasodilating PGs into the coronary effluent, we measured the concentration of both prostacyclin and PGE_2. Prostacyclin levels were measured by measuring its stable metabolite $PGF_{1\alpha}$ (Fig. 5). The basal concentration of 6-keto-$PGF_{1\alpha}$ in the perfusate was significantly greater in aged rats than young rats (71±8.9 pg/ml in the young rat ($n=6$); 161±45 pg/ml in the aged rat ($n=5$)). In the aged rat, urotensin II increased the concentration of 6-keto-$PGF_{1\alpha}$ (peak level: 1234±284 pg/ml) within 5 min and this level decreased to 535±91 pg/ml after 10 min. In the young rat, 6-keto-$PGF_{1\alpha}$ significantly increased to 1159±62 pg/ml after stimulation with urotensin II. Diclofenac (10 μM) abolished the urotensin II-induced increase in prostacyclin both in young and aged rats.

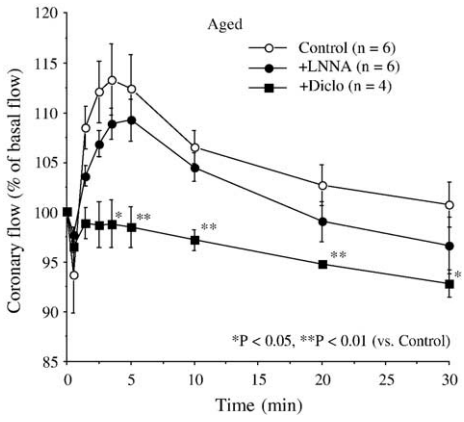

Fig. 4. Effect of nitric oxide synthase inhibitor L-NNA and cyclooxygenase inhibitor diclofenac on the urotensin II-induced coronary vasodilatation in Langendorff-perfused aged rat hearts. Continuous infusion of L-NNA (10 μM) and/or diclofenac (10 μM) started 10 min before and continued during the application of urotensin II by using a syringe pump. Results are expressed as means±S.E.M. *$P<0.05$, **$P<0.01$ vs. urotensin II alone.

Release of another vasodilating PGE_2 was also stimulated by urotensin II (Fig. 6). The concentration of PGE_2 in the young rat increased from 1.1±0.1 pg/ml to a peak level of 293±35 pg/ml within 3 min of urotensin II application (100 nM). The release of PGE_2 was also stimulated in the aged rat; aging did not alter the response of PGE_2 (Fig. 6). Diclofenac (10 μM) significantly inhibited the urotensin II-induced production of PGE_2.

3.5. Effects of urotensin II on the release of 8-isoprostane into the coronary effluent

8-Isoprostane is a biologically active metabolite of arachidonic acid; we measured the levels of 8-isoprostane, as it is possibly a marker of free radical production. Urotensin II stimulated the release of 8-isoprostane into coronary effluent. The concentration of 8-isoprostane increased to about double the basal value 5 min after stimulation (39.8±3.6 pg/ml in young rats, 40.2±4.4 pg/ml in aged rats). However, we found no age-related changes in the basal and peak concentrations of 8-isoprostane (Fig. 7). In order to examine whether cyclooxygenase was involved in the urotensin II-induced increase in

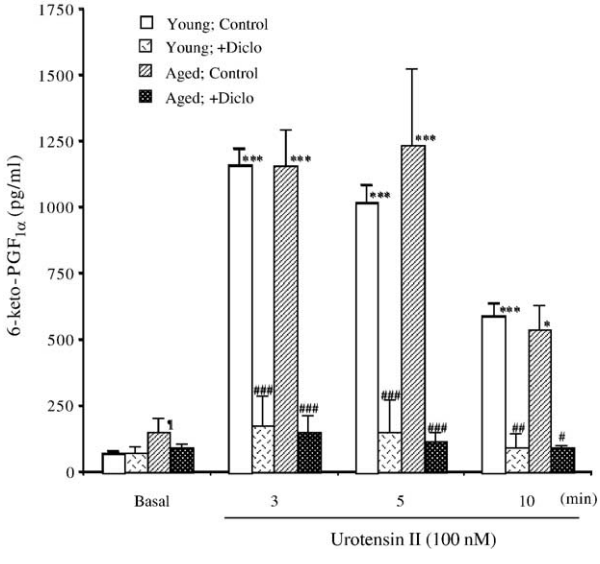

Fig. 5. Effect of urotensin II on the concentration of prostacyclin released into the coronary effluent of young and aged rats. The concentration of prostacyclin was measured as its stable metabolite (6-keto-$PGF_{1\alpha}$) by enzyme-immunoassay. Control: urotensin II alone, +Diclo: in the presence of diclofenac (10 μM). Results are expressed as means±S.E.M. ($n=5$–6). ¶$P<0.05$ vs. Young (Basal); *$P<0.05$, ***$P<0.001$ vs. basal value; #$P<0.05$, ##$P<0.01$, ###$P<0.001$ vs. control at each time point.

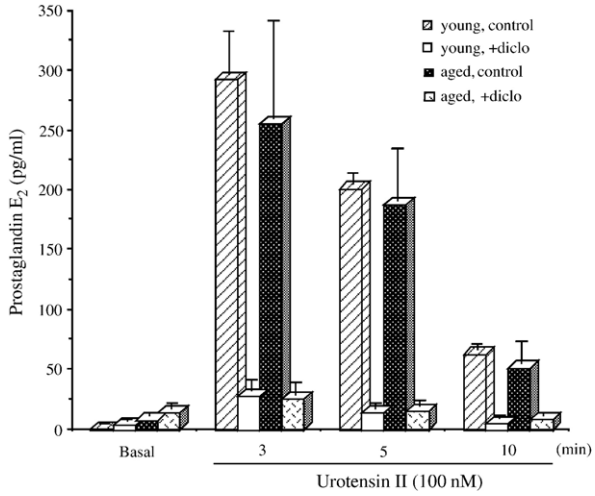

Fig. 6. Effect of urotensin II on the concentration of PGE_2 released into coronary effluent of young and aged rats. The concentration of PGE_2 was measured by enzyme-immunoassay. Control: urotensin II alone, +Diclo: in the presence of diclofenac (10 μM). Results are expressed as means±S.E.M. (n=4–6). There was no difference in the response of young and aged rats.

8-isoprostane, the concentration of 8-isoprostane in the presence of diclofenac was measured. Diclofenac significantly attenuated the production of 8-isoprostane (Fig. 7).

3.6. Effects of urotensin II on the release of NO into the coronary effluent

The administration of urotensin II to the coronary perfusate rapidly increased the concentration of NO_x in the young rat (Fig. 8). The level of NO_x in the effluent of the young heart increased from a basal value of 0.44±0.24 μmol/l to 1.25±0.67 μmol/l 3 min after injection of urotensin II. The increase in NO_x concentration as a response to urotensin II was not affected by

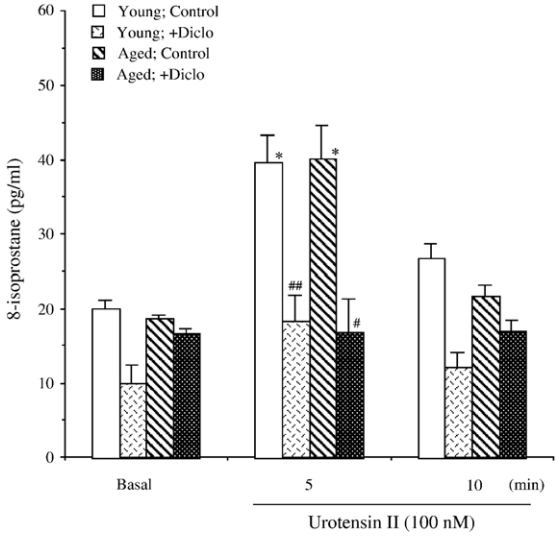

Fig. 7. Effect of urotensin II on the concentration of 8-isoprostane released into the coronary effluent of young and aged rats. The concentration of 8-isoprostane was measured by enzyme-immunoassay. Control: urotensin II alone, +Diclo: in the presence of diclofenac (10 μM). Results are expressed as means±S.E.M. (n=4–8). $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. control (5 min); $^{*}P<0.05$ vs. basal value.

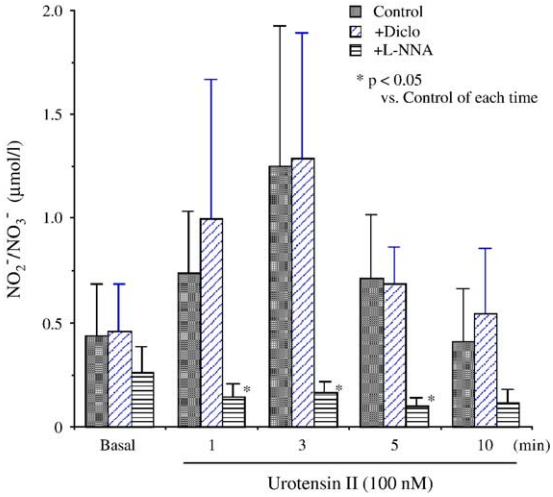

Fig. 8. Effect of urotensin II on the concentration of NO produced in young rat coronary arteries. After infusion of urotensin II, the concentrations of nitrate plus nitrite (NO_x) in the coronary effluent were measured fluorometrically. Control: urotensin II alone, +Diclo: in the presence of diclofenac (10 μM), +L-NNA: in the presence of L-NNA (10 μM). Results are expressed as means±S.E.M. (n=4–7). $^{*}P<0.05$ vs. control at each time point.

diclofenac, but was thoroughly inhibited by L-NNA. Interestingly, urotensin II did not enhance the production of NO_x in the aged rat (Fig. 9), suggesting little involvement of NO in the urotensin II-induced vasodilatation in the aged heart.

4. Discussion

Urotensin II has a strong vasopressor effect in isolated blood vessels from a variety of mammalian species such as monkeys, pigs, dogs, rabbits, mice and humans (Ames et al., 1999; Douglas et al., 2000). In contrast, we have shown here that the predominant effect of urotensin II in Langendorff-perfused rat hearts was vasodilatation of coronary arteries in both young and senescent rats (Fig. 1). The administration of urotensin II into

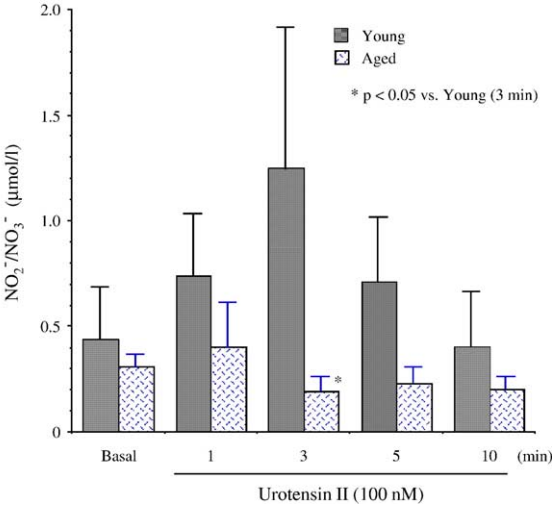

Fig. 9. Comparison of the urotensin II-induced release of NO in young and aged coronary arteries. After infusion of urotensin II, the concentrations of nitrate plus nitrite (NO_x) in the coronary effluent were measured fluorometrically. Results are expressed as means±S.E.M. (n=6–7). $^{*}P<0.05$ vs. young rats.

the coronary artery caused a sustained vasodilatation and the responses were not significantly different between young and aged hearts. These results are consistent with previous reports that have indicated that the main cardiovascular action of urotensin II is vasodilatation in small pulmonary and abdominal resistance arteries in the human (Stirrat et al., 2001), in coronary and renal arteries of the rat (Bottril et al., 2000; Katano et al., 2000; Zhang et al., 2003) and reduces blood pressure in conscious rats (Gardiner et al., 2001).

Although urotensin II was originally reported to be one of the most potent vasoconstrictors, the vasoconstricting effects of urotensin II that vary among different tissues and species have now been elucidated (Douglas et al., 2000). These differences may be attributed to differences in the expression of urotensin II receptors and to the absence of spare receptors combined with slow dissociation of urotensin II from these receptors (Douglas et al., 2004). In addition, the contractile response to urotensin II can be modulated or masked by endothelial cell-derived vasodilating substances (Ishihata et al., in press). Several kinds of endothelium-derived vasorelaxing factors are considered to be involved in urotensin II-induced vasodilatation. For example, urotensin II caused a concentration-dependent relaxation of 5-hydroxytryptamine (5-HT)-precontracted arteries, which was abolished by a NO synthase inhibitor (N^G-nitro-L-arginine methyl ester hydrochloride) or by removal of endothelium (Bottril et al., 2000). The endothelial vasodilating factors can reduce or mask the vasoconstricting effect of urotensin II on smooth muscle. In other words, the net effects of vasoconstriction and vasodilation by urotensin II may represent the overall vascular response. This may explain the conflicting results obtained by Gray et al. (2001) that demonstrated urotensin II-induced vasoconstriction in rat coronary arteries.

In this study, we applied human urotensin II to our rat preparations and this might have affected the coronary response. However, it has been shown that the binding characteristics of urotensin II isopeptides to their receptors are indistinguishable among species. Urotensin II isopeptides share conserved, cyclic hexapeptides (CFWKYC) over a wide range of species and contain a disulfide bond between two cysteine residues. The cyclic hexapeptides exhibit full agonistic activity for the urotensin II receptor and show a vasoconstricting effect in isolated vessels (Bottril et al., 2000; Katano et al., 2000; Gray et al., 2001; Stirrat et al., 2001; Zhang et al., 2003; Douglas et al., 2004). Therefore, the difference in the coronary arterial effect of urotensin II (vasodilatation vs. vasoconstriction) is not due to the application of human urotensin II in the rat.

In order to elucidate the role of coronary endothelial cells in urotensin II-induced vasodilatation, experiments where the endothelial cells have been removed with detergent may be interesting. Although there are some reports of endothelial cell removal with Triton X-100 (Fort and Lewis, 1993; Hassanbad et al., 1998; Kennedy et al., 1999), strict experimental conditions are required to denude endothelial cells perfectly without impairing cardiac muscle and vascular smooth muscle cells, as it is known that treatment with Triton X-100 increases cardiac arrhythmia in ischemia–reperfusion (Hassanbad et al., 1998). Therefore, we chose to treat the vessels using pharmacological agents in preference to detergents.

This study has shown that urotensin II-induced coronary vasodilatation in rats was mediated through increased production of endothelium-derived relaxing factors. Because aging affects endothelial synthesis of various factors, we investigated which endothelial factors were involved in the urotensin II-induced increase in coronary flow in both young and aged rats. Urotensin II increased the concentration of 6-keto-$PGF_{1\alpha}$ and PGE_2 in the coronary effluent and aging did not alter these responses (Figs. 5 and 6). Diclofenac (10 μM), a cyclooxygenase inhibitor, significantly inhibited the urotensin II-induced production of 6-keto-$PGF_{1\alpha}$ and PGE_2 (Figs. 5 and 6) and attenuated the urotensin II-induced coronary vasodilatation in both young and aged rats (Figs. 3 and 4). These results suggest that prostacyclin and PGE_2 are involved in the urotensin II-induced coronary vasodilatation in both young and aged rat hearts. On the other hand, a NO synthase inhibitor L-NNA reduced the urotensin II-stimulated increase in coronary flow in the young rat but not in the aged rat (Figs. 2 and 4), suggesting an age-dependent difference in the contribution of NO in urotensin II-induced vasodilatation. To further examine the role of NO in urotensin II-induced coronary vasodilatation, NO concentration was measured directly in the coronary effluent. We showed that the concentration of NO in the coronary effluent increased in response to urotensin II in the young, but not in the aged rat (Fig. 9). Taken together, both NO and PGs (prostacyclin and PGE_2) are very likely involved in the urotensin II-induced coronary vasodilatation in young rats, while prostacyclin and PGE_2 are primarily responsible for the urotensin II-induced coronary dilatation in the aged rat. These findings are consistent with previous reports (Katano et al., 1993; Amrani et al., 1996), which indicate that aging is one of the pathophysiological factors involved in impaired production of NO. Lamontagne et al. (1992) has indicated that prostacyclin could play an important role in vasodilatation when the NO pathway was impaired. We have recently shown that prostacyclin may play a crucial role as a vasodilating factor that compensates for the reduced production of NO in aged rat coronary arteries (Ishihata et al., 1999). Although the production of NO was reduced in the aged rat, it may be possible that aged endothelial cells produced vasodilating PGs (prostacyclin and PGE_2) as a compensatory mechanism for reduced production of NO. In fact, the concentration of prostacyclin and PGE_2 in the aged rat coronary effluent increased to a level almost equivalent to the young rat (Figs. 5 and 6). These results support the hypothesis that urotensin II increased coronary flow in the aged rat through increased production of vasodilating PGs.

Another endothelium-dependent vasorelaxing factor is EDHF (Mombouli and Vanhoutte, 1997), which plays an important role especially in pathophysiological conditions. For example, EDHF-mediated relaxation in the renal artery of hypercholesterolemic rabbits is enhanced (Moroe et al., 2004). The enhanced EDHF component in the renal artery appeared to serve as a compensatory mechanism in the maintenance of vasorelaxation. Therefore, it would be possible that urotensin II might cause vasodilatation via EDHF. In this study, however,

combined infusion of L-NNA and diclofenac completely blocked the vasodilator response to urotensin II, and instead of vasodilatation a weak vasoconstriction was observed. These results strongly suggest that EDHF may not be involved in the urotensin II-induced vasodilatation in rat coronary arteries.

In the young rat, diclofenac strongly inhibited urotensin II-induced vasodilatation. It was thought that blocking the cyclooxygenase pathway, but leaving the other NO pathway intact, would result in more moderate urotensin II-induced vasodilatation. This does not seem to be the case in our current experiments. It could be possible that the use of diclofenac (10 μM) in our experiments caused inhibition of cyclooxygenase and may have had some nonspecific effect on vascular smooth muscle contractility. Alternatively, there may possibly be an interaction between cyclooxygenase and the NO-guanylate cyclase pathway (Laemmel et al., 2003; Merkus et al., 2004). Although NO production was uninhibited by diclofenac in our present study, we have not as yet determined whether the activity of guanylate cyclase is nonspecifically altered by diclofenac.

Impairment of endothelium-dependent relaxation may partly result from NO inactivation by oxygen-derived free radicals (Adeagbo and Triggle, 1993; Ohara et al., 1993). To determine if the NO inactivation was accelerated by increased oxygen-derived free radicals in the aged rat heart, we measured the concentration of 8-isoprostane as a possible marker of free radical production. It has been shown that isoprostanes are metabolized from arachidonic acid via a free radical-mediated reaction. For example, in a model of ischemia–reperfusion where the increased oxygen-free radicals stimulated lipid peroxidation, it was noted that formation of 8-isoprostane was induced mainly in coronary endothelium (Delanty et al., 1997; Wilson et al., 1999; Mehlhorn et al., 2003). However, we have shown that aging did not alter the release of 8-isoprostane into the coronary effluent (Fig. 7). In accordance with our present results, Brunetti et al. (2004) reported that there was no significant difference in basal release of 8-isoprostane in the brain synaptosome of the young or aging rat. Recent studies have indicated an existence of a new pathway of free radical-independent and cyclooxygenase-dependent formation of 8-isoprostane in renal tissue, isolated vascular cells (Jourdan et al., 1999) and perfused lung (Montuschi et al., 1999). In addition, activation of phospholipase A_2 increased a release of total 8-isoprostane in the umbilical vein and the rabbit ear artery (Sametz et al., 2000a,b). Therefore, it may be possible that urotensin II-induced formation of 8-isoprostane is mediated through activation of cyclooxygenase, and that this pathway is also involved in the production of prostacyclin and PGE_2. Actually, pretreatment with diclofenac significantly attenuated urotensin II-induced 8-isoprostane production (Fig. 7).

We have demonstrated here that urotensin II could stimulate the formation of 8-isoprostane and the release of prostacyclin/PGE_2 in the Langendorff-perfused rat heart, and that aging did not modify these responses. The lack of age-related changes in the basal level and urotensin II-stimulated production of prostacyclin, PGE_2 and 8-isoprostane suggests that cyclooxygenase activity is maintained in the aged rat, which contrasts the decreased NO formation in aged coronary endothelium.

Although 8-isoprostane production increased 5 min after urotensin II application, coronary flow was not attenuated (Fig. 1). This may be due to the relatively low concentration of 8-isoprostane and counteractivity of vasodilating NO and PGs. The concentration of 8-isoprostane in the coronary effluent was around 40 pg/ml, while the EC_{50} value was ≈700 pg/ml and the threshold was ≈100 pg/ml (Sametz et al., 2000a,b). In addition, the production of 8-isoprostane was primarily dependent on cyclooxygenase (Fig. 7) and was completely inhibited by diclofenac. This may possibly explain why severe coronary vasoconstriction was not induced even when vasodilating PGs were inhibited by diclofenac.

Although the main effect of urotensin II on the coronary arteries was vasodilatation, urotensin II also very transiently decreased coronary flow. The possible reason for this transient decrease in coronary flow may be endothelial O_2^- formation by activating NADPH oxidase (Oudot et al., 2003), which in turn leads to a decrease in NO bioavailability, and possibly a vasoconstrictive effect of urotensin II on smooth muscle.

In conclusion, this study has revealed that aging did not attenuate the vasodilating effects of urotensin II on coronary arteries. Although aging diminished the urotensin II-induced production of NO, aged coronary arteries released vasodilating PGs (prostacyclin and PGE_2) at almost equivalent levels to young rats. These results indicate that prostacyclin and PGE_2 may play an important role in maintaining the coronary vasodilating response to urotensin II in aged rats.

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