Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 467 (2003) 151–161
www.elsevier.com/locate/ejphar

Endothelium-derived prostaglandin H_2 evokes the stretch-induced contraction of rabbit pulmonary artery

Maki Saito[a], Yoshiyuki Tanabe[a], Ichiro Kudo[b], Koichi Nakayama[a,*]

[a] Department of Pharmacology, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka-city, Shizuoka 422-8526, Japan
[b] Department of Health Chemistry, School of Pharmaceutical Sciences, Showa University, Shinagawa, Tokyo 142-8555, Japan

Received 26 August 2002; received in revised form 28 February 2003; accepted 5 March 2003

Abstract

Stretch-induced contraction of rabbit pulmonary artery depends on endothelium-derived vasoactive prostanoids. We investigated which prostanoid(s) was responsible for the stretch-induced contraction of the artery, and whether integrin was involved in this mechanotransduction process. Stretch increased productions of untransformed prostaglandin H_2, prostaglandin E_2, prostaglandin $F_{2\alpha}$, and thromboxane A_2 in the pulmonary artery with intact endothelium. A blocking peptide for integrins (RGD peptide) significantly inhibited productions of thromboxane A_2 and prostaglandin $F_{2\alpha}$, but the peptide did not affect productions of untransformed prostaglandin H_2 and prostaglandin E_2, as well as contraction in response to stretch. SQ29,548, a prostaglandin H_2/thromboxane A_2 receptor antagonist, inhibited the contractile response to not only stretch but also exogenous prostaglandin H_2. Acetylcholine (up to 30 μM) also contracted the artery in an endothelium-dependent manner. Ozagrel (10 nM–1 μM), an inhibitor of thromboxane synthase, abolished the production of thromboxane A_2, in response to both stretch and acetylcholine, whereas the inhibitor mostly inhibited acetylcholine-induced contraction, but it did not suppress stretch-induced contraction. The results suggested that prostaglandin H_2 and thromboxane A_2, either released from endothelium by mechanical stretch or by acetylcholine, produced contraction of rabbit pulmonary artery in a RGD-independent manner.

Keywords: Pulmonary artery; Stretch; Contraction; Prostaglandin H_2; Integrin

1. Introduction

Mechanical stimuli, for instance, stretch and intraluminal pressurization, which might mimic an acute and/or chronic change in hemodynamic forces such as blood pressure and blood flow, induce a variety of responses including contraction, activation of various protein kinases and cationic channels, production of vasoactive substances, gene expression, and proliferation in the cardiovascular system. We previously reported that cerebral, coronary, and pulmonary arteries of various animal species including rabbit, rat, and dog were particularly sensitive to mechanical stretch, and that the stimulus produced contractions (Nakayama, 1982; Nakayama et al., 1986, 1989, 1997, 2003; Nakayama and Tanaka, 1993; Masumoto et al., 1997; Obara et al., 2001, 2002).

As to the mechanism of stretch-induced contraction of rabbit pulmonary artery, however, we had considered that the contractile response of rabbit pulmonary artery was primarily dependent on thromboxane A_2 and/or an increase in the ratio thromboxane A_2/prostaglandin I_2 released in an endothelium-dependent manner (Nakayama et al., 1997).

According to our previous report (Tanabe et al., 2000), the stretch activated tyrosine phosphorylation of focal adhesion kinase (FAK) in rabbit pulmonary artery tissues, and the pulmonary artery-derived cultured smooth muscle cells, as well as the endothelial cells. Since FAK has been considered to associate with cytoplasmic domain of integrin, it might be possible that the interaction of integrin with extracellular matrix is involved in the mechanotransduction of the pulmonary artery. However, it has still remained to be solved whether endothelium-dependent production of prostanoids is regulated via the interaction of integrin with extracellular matrix. Furthermore, in addition to thromboxane A_2, it was reported that various prostanoids such as

* Corresponding author. Tel.: +81-54-264-5691; fax: +81-54-264-5696.
E-mail address: nakyamk@ys7.u-shizuoka-ken.ac.jp (K. Nakayama).

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01569-3

prostaglandin $F_{2\alpha}$, prostaglandin E_2, prostaglandin D_2, and prostaglandin H_2 also acted as vasoconstrictors, on the rabbit pulmonary artery (Barnes and Liu, 1995). Thus, we aimed to clarify which prostanoid(s) was responsible for the stretch-induced contraction of the rabbit pulmonary artery, and whether integrin pathway(s) in endothelium was involved in the production of these prostanoids in the artery.

Acetylcholine at a high concentration (~ 10 μM) also has been reported to induce contraction of rabbit pulmonary artery, which is mediated by thromboxane A_2 (Altiere et al., 1986; Buzzard et al., 1993; Nakayama et al., 1997). Therefore, the contractile response to acetylcholine of the rabbit pulmonary artery was compared with that to stretch.

The results presented here suggest that stretch augments productions of prostanoids such as thromboxane A_2, prostaglandin $F_{2\alpha}$, prostaglandin E_2, untransformed prostaglandin H_2, and prostaglandin I_2 in an endothelium-dependent manner. The former two productions (thromboxane A_2 and prostaglandin $F_{2\alpha}$) were dependent on Arg-Gly-Asp (RGD) peptide-sensitive integrins, whereas the latter three (prostaglandin E_2, untransformed prostaglandin H_2, and prostaglandin I_2) were independent of these integrins. Of these prostanoids, moreover, the untransformed prostaglandin H_2 was primarily responsible for the stretch-induced contraction of rabbit pulmonary artery, whereas thromboxane A_2 was preferentially involved in the acetylcholine-induced contraction of the artery.

2. Materials and methods

2.1. Preparations of rabbit pulmonary arteries

Experimental animals were treated according to Guideline for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. Japanese White rabbits of either sex (2.5–3.5 kg) were given heparin (1000 units/kg) (Nihon Schering, Osaka, Japan) via an ear vein and anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The rabbits were then sacrificed by bleeding from the common carotid arteries. After thoracotomy, the lungs and heart were removed as a whole, and immersed in Tyrode's solution consisting of the following composition (in mM): NaCl 158.3, KCl 4.0, $CaCl_2$ 2.0, $MgCl_2$ 1.05, $NaHCO_3$ 10.0, NaH_2PO_4 0.42, and glucose 5.6. The isometric high K^+ (80 mM KCl) solution was prepared by replacing the NaCl with an equimolar amount of KCl. The solution was bubbled with 95% O_2 and 5% CO_2 during preparation of the pulmonary artery at a room temperature (around 25 °C). The first branch of the intrapulmonary lobular artery having an outer diameter of about 2 mm, which was about 1.5 cm distant from the hilum of right or left lung, was carefully isolated. The pulmonary artery tissue having intact endothelium was cleared of fat and connective tissue under a dissection microscope. Then the artery was cut into ring segments about 4 mm wide and 2–3 mg in wet weight for biochemical experiments, except that the ring with 2 mm width was prepared for functional experiments to assess the effects of exogenous prostaglandin E_2 and prostaglandin H_2 on the contractility of the artery. In some preparations, the endothelium was removed by gentle rubbing with moist absorbent-cotton pledget, and the absence of acetylcholine (30 nM)-induced relaxation of the pulmonary artery preconstricted with 100 nM prostaglandin $F_{2\alpha}$ was checked to confirm a successful removal of endothelium.

2.2. Recording of isometric tension and experimental protocol for stretch activation of pulmonary artery

Two L-shaped tungsten wires (300 μm in diameter), one was attached to a micromanipulator and the another one was attached to a force transducer (T7-30-240, Orientec, Tokyo, Japan), were inserted through the lumen of the ring segment. Each segment was maintained in organ bath filled with 2 ml of Tyrode's solution at 37 °C with gassing of 95% O_2 and 5% CO_2. All signals from the force transducer, amplified with a biological amplifier (6M82, GE Marquette Medical Systems Japan, Tokyo, Japan), were recorded and analyzed by use of MacLab® System (AD Instruments, Sydney, Australia). An initial muscle length (Li = 100%), i.e., the unloaded condition of ring, was determined by adjusting a distance between the parallel wires at which detectable passive tension began to appear. The length of the ring segment was calculated by the following formula as reported previously (Nakayama et al., 1997): $L = 2a + b(\pi + 2)$, where a = distance (in mm) between the inner edges of two wires that were parallel to each other; b = diameter of the tungsten wire. For the ring segments, L is the distance around the inside edge of the ring, i.e., inside circumference assuming the segment to be cylindrical in shape. Bathing solution was replaced with fresh Tyrode's solution every 15 min during equilibration period for 1 h, then the ring segment was repeatedly stretched to 1.8 Li (180% of initial muscle length) for 5 min with at least 15 min interval until the reproducible amplitude of tension appeared (usually three times). The amount of stretch was adjusted with the micromanipulator, and the rate of stretch was kept constant at approximately 0.5 mm/s. For quantitative analysis of the stretch-induced contraction, the area under the tension curve above control resting release for the 5 min period was measured by NIH Image software (ver. 1.62, National Institutes of Health, Bethesda, MD, USA). The passive increase in tension elicited by stretch was defined as the response after administration of papaverine (100 μM), which could totally eliminate the active tension of the artery. The active mechanical response during stretch was obtained by subtracting the area corresponding to the increase in passive tension from the total circumscribed area, and expressed as percent of that induced by stretch

before the treatment with any drugs. The dimension of unit of the contraction was product of force and time.

Similar concentration–response curves for cumulative applications of acetylcholine up to 30 μM were reproduced at least twice, and we determined the effects of drugs or vehicle on second round of acetylcholine-induced contraction. Data were expressed as percent of contraction produced by 80 mM KCl (=100%). ED_{50} values were determined by probit analysis.

In the functional experiments to assess the effects of exogenous prostanoids or acetylcholine, the arteries of 2 mm width in the organ bath were allowed to equilibrate for at least 60 min under an passive load of 9.8 mN, at which the maximum contraction induced by 80 mM KCl solution was observed.

2.3. Measurement of production of prostanoids

The artery ring of 4 mm width at Li was incubated for 5 min in 2 ml of fresh Tyrode's solution (basal), then stretched to 1.8 Li or treated with 30 μM acetylcholine for 5 min in another 2 ml of fresh Tyrode's solution. Production of each prostanoids was measured before and after mechanical (stretch) or pharmacological (acetylcholine) stimulations. Then, the data obtained were analyzed by paired comparison. Bathing solution of each condition was collected and subjected to enzyme immunoassay. Concentrations of thromboxane B_2 (a stable metabolite of thromboxane A_2), prostaglandin $F_{2\alpha}$, prostaglandin E_2, prostaglandin D_2-methoxime (a stable metabolite of prostaglandin D_2), 6-keto prostaglandin $F_{1\alpha}$ (a stable metabolite of prostaglandin I_2) (pg/min/mg wet tissue) in the bathing solution were measured directly by use of commercially available enzyme immunoassay kits (#519031, 516011, 514010, 512011, and 515211, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions. Specificity of antibodies included in the kit have been examined extensively by the company. No appreciable interference of each assay with Tyrode's solution, drugs, and chemicals used in the experiments was observed. Prostaglandin H_2 is an unstable molecule that is converted to prostaglandin $F_{2\alpha}$ by a mild reducing agent, such as Tin (II) chloride ($SnCl_2$) (Hamberg et al., 1974). Therefore, to estimate the untransformed prostaglandin H_2 released, we calculated the difference between prostaglandin $F_{2\alpha}$ in bathing solution of arterial preparations in the presence or absence of $SnCl_2$ (1.05 mM) as previously described by Camacho et al. (1998).

In a separate series of experiments, we checked the blocking action of RGD peptide on the interaction of integrin with extracellular matrix (Pierschbacher and Ruoslahti, 1987), i.e., adhesion of cultured rabbit smooth muscle cells derived from pulmonary artery to fibronectin- or gelatin-coated plastic multiwell plate. Of the concentrations tested (1–100 μM), Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP; RGD peptide) at 100 μM maximally inhibited the cell-adhesion, but Gly-Arg-Gly-Glu-Ser-Pro (GRGESP; RGE peptide), an inactive control, did not affect it (data not shown). Thus, in the present study, we used each peptide at 100 μM.

2.4. Drugs and chemicals

The following drugs were used: Acetylcholine hydrochloride, N^G-nitro-L-arginine (L-NNA) (Sigma-Aldrich, St. Louis, MO, USA); SQ29,548 ([1*S*-[1α, 2α(*Z*), 3α, 4α]]-7-[3-[2-[(phenylamino) carbonyl] hydrazino] methyl-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid) (Research Biochemicals International, Natick, MA, USA); GRGDSP (RGD peptide) (glycyl-arginyl-glycyl-asparatyl-seryl-proline), GRGESP (RGE peptide) (glycyl-arginyl-glycyl-glutamyl-seryl-proline) (GIBCO BRL Life Technologies, Gaithersburg, MD, USA); prostaglandin $F_{2\alpha}$, prostaglandin E_2, and prostaglandin H_2 (Cayman Chemicals); ozagrel [(*E*)-3-[4-(imidazoylmethyl)phenyl]-2-propenoic acid] (OKY-046; Kissei, Matsumoto, Japan); BQ-123 [cyclo (D-Asp-Pro-D-Val-Leu-D-Trp)] (Novabiochem, Darmstadt, Germany); esculetin (Extrasynthése, Genay, France); WEB2086 (3-[4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3, 2-*f*] [1, 2, 4]triazolo[4, 3-α][1, 4]diazepin-2-yl]-1-propanone) was kindly provided by Dr. M. Miwa at our university. All other reagents were purchased from Wako (Osaka, Japan) or Sigma-Aldrich. SQ 29,548, prostaglandin $F_{2\alpha}$, prostaglandin E_2, and prostaglandin H_2 were dissolved in ethanol. All drugs were further diluted with Tyrode's solution. The molar concentration (M) in the bathing solution is given for all drugs used.

2.5. Statistics

The data are expressed as the mean ± S.E.M. Statistical analyses were done by paired Student's *t*-test. To determine whether responses differed significantly among groups, we performed Sheffe's *F* test or Fisher's Protected Least Significant Difference test after analysis of variance (ANOVA). Differences were considered significant when the *P* value was less than 0.05.

3. Results

3.1. Mechanical responses and productions of prostanoids to stretch in rabbit pulmonary artery

Fig. 1 shows typical contractile responses of a pair of ring segments of rabbit pulmonary artery in the presence (left panel) or absence (right panel) of endothelium to stretch. The stretch was given to the artery by the standard procedure described in Section 2, i.e., a rate of stretch of 0.5 mm/s, an amount of stretch of 1.8 Li (180% of initial muscle length), and a stimulus period of 5 min with at least 15-min intervals. An initial rise in tension occurred with stretch and

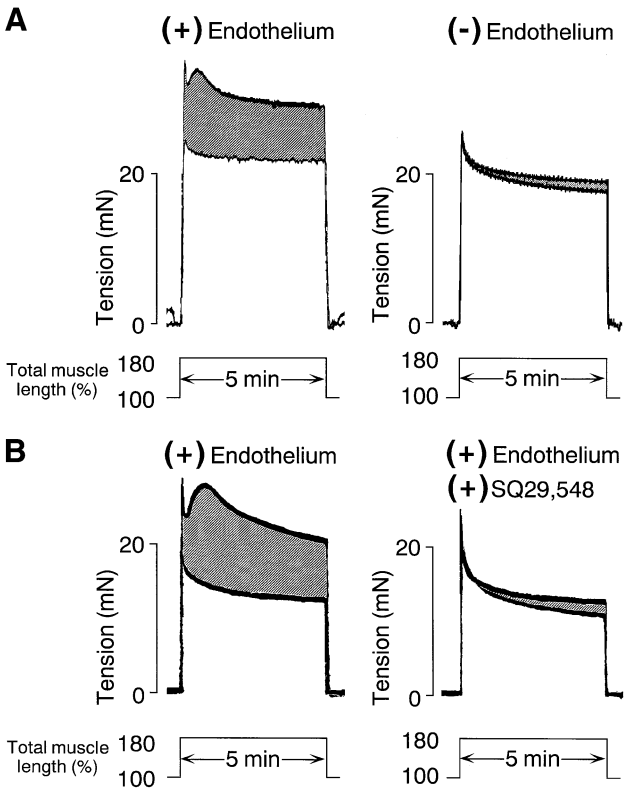

Fig. 1. Mechanical response of rabbit pulmonary artery in response to stretch: their dependence on endothelium and abolition by SQ29,548. (A) Actual tracings show the isometric tension of rabbit pulmonary arteries with ((+) Endothelium, left) and without ((−) Endothelium, right) endothelium in response to stretch to 1.8 Li (180% of initial muscle length), and a stimulus period of 5 min. Active tension was superimposed on passive tension obtained after administration of 100 μM papaverine. Hatched areas of the mechanograms represent the active tension induced by the stretch for 5 min. (B) Actual tracings show the stretch-induced contraction of rabbit pulmonary artery with intact endothelium before and after application of 100 nM SQ29,548, a thromboxane A_2/prostaglandin H_2 receptor antagonist. Active tension was measured as mentioned in (A).

the subsequent fall at the completion of the stretch was followed within 10 s by a delayed contraction that reached maximum about a minute after application of stretch. The hatched area of the mechanogram showed the active tension which was superimposed on the passive tension obtained after repeated stretches in the solution containing 100 μM papaverine (Fig. 1A, left). The contraction in response to stretch was strongly attenuated when the endothelium was mechanically removed (Fig. 1A, right), indicating the endothelium-dependent contractile response to stretch.

Since the mechanical responses to stretch were thoroughly eliminated by indomethacin (10 μM) or aspirin (10 μM), cyclooxygenase inhibitors, or SQ29,548 (100 nM), a thromboxane A_2/prostaglandin H_2 receptor (TP-receptor) antagonist, we considered that the contraction of rabbit pulmonary artery produced by stretch was evoked by endothelium-derived cyclooxygenase products (Nakayama et al., 1997), including thromboxane A_2. In the present study, we reconfirmed the blocking effect of SQ29,548 on the stretch-induced contraction of the pulmonary arteries (Fig. 1B). Thus, we again indicated an implication of TP-receptor pathway in the stretch-induced contraction.

We also examined the effect of WEB2086 (100 nM), a receptor antagonist of platelet-activating factor (PAF), esculetin (10 μM), a 5- and 12-lipoxygenase inhibitor, and BQ123, an endothelin A receptor (ET_A) antagonist on the

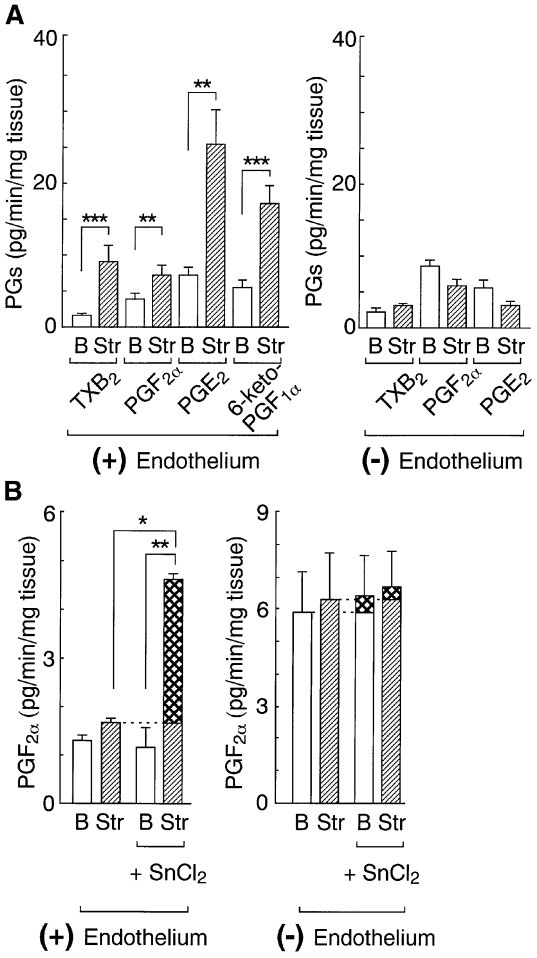

Fig. 2. Augmented production of endothelium-derived prostanoids in rabbit pulmonary arteries in response to stretch. (A) The amount of prostanoids produced before (open columns) and after (hatched columns) stretch was indicated as pg/min/mg wet tissue. Each column with bar represents the mean ± S.E.M. of the following numbers of independent preparations. (+) Endothelium, endothelium-intact arteries: Thromboxane (TX) A_2 (as TXB_2, $n=16$), prostaglandin (PG) $F_{2\alpha}$ ($PGF_{2\alpha}$, $n=32$), prostaglandin E_2 (PGE_2, $n=5$), and prostaglandin I_2 (as 6-keto $PGF_{1\alpha}$, $n=5$); (−) Endothelium, endothelium-removed arteries: thromboxane A_2 (as TXB_2, $n=4$), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, $n=4$), and prostaglandin E_2 (PGE_2, $n=4$). $^{**}P<0.01$, $^{***}P<0.001$ vs. each corresponding value. B: basal release, and Str: stretch. (B) Untransformed prostaglandin H_2 produced by stretch. The amount of untransformed prostaglandin H_2 released in the bathing solution was estimated by measuring prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) increased in the presence of 1.05 mM $SnCl_2$ as described in Section 2. Estimated release of untransformed prostaglandin H_2 was indicated as cross-hatched area in each column. Data represent the mean ± S.E.M. of the following numbers of independent preparations. (+) Endothelium, endothelium-intact arteries ($n=4$); (−) Endothelium, endothelium-removed arteries ($n=4$). $^{*}P<0.05$, $^{**}P<0.01$ vs. each corresponding value. B: basal release, and Str: stretch.

stretch-induced contraction of the rabbit pulmonary artery. None of them altered stretch-induced contraction (data not shown), suggesting that PAF, lipoxygenase products, or endothelin play a minor role in stretch-induced contraction.

In accordance with the idea, the releases of various prostanoids, including thromboxane A_2 (measured as thromboxane B_2), prostaglandin $F_{2\alpha}$, prostaglandin E_2, prostaglandin I_2 (measured as 6-keto prostaglandin $F_{1\alpha}$) and untransformed prostaglandin H_2 (measured as increased prostaglandin $F_{2\alpha}$ in the presence of $SnCl_2$), were significantly increased in the artery with intact endothelium (Fig. 2A and B, (+) Endothelium). For instance, in the endothelium-intact arteries, basal release of thromboxane A_2 was 1.71 ± 0.35 pg/min/mg wet tissue and the release after the stretch was 9.26 ± 2.06 pg/min/mg wet tissue (each $n = 16$, $P < 0.001$) (Fig. 2A, (+) Endothelium), which was roughly fivefold increase in the production of thromboxane A_2. Likewise, stretch significantly augmented the production of prostaglandin $F_{2\alpha}$ about twofold (basal, 4.00 ± 0.67 pg/min/mg wet tissue; after stretch, 7.20 ± 1.48 pg/min/mg wet tissue, each $n = 32$, $P < 0.01$), that of prostaglandin E_2 about threefold (basal, 7.23 ± 1.19 pg/min/mg wet tissue; after stretch 25.5 ± 4.69 pg/min/mg wet tissue, each $n = 15$, $P < 0.01$), and that of prostaglandin I_2 about threefold (basal, 5.43 ± 1.24 pg/min/mg wet tissue; after stretch, 16.81 ± 2.50 pg/min/mg wet tissue, each $n = 5$, $P < 0.001$), respectively (Fig. 2A, (+) Endothelium). In contrast, production of prostaglandin D_2 in the artery with intact endothelium was undetectable (< 0.4 pg/min/mg wet tissue) in any conditions.

The stretch-induced production of prostaglandin $F_{2\alpha}$ (4.61 ± 0.12 pg/min/mg wet tissue) in the presence of $SnCl_2$ was significantly larger in the endothelium-intact arteries than that produced in the absence of $SnCl_2$ (1.64 ± 0.10 pg/min/mg wet tissue) (each $n = 4$, $P < 0.05$). Thus the estimated amount of untransformed prostaglandin H_2 was 2.97 ± 0.87 pg/min/mg wet tissue (Fig. 2B). However, the untransformed prostaglandin H_2 was not detected when the arteries were incubated under the unstretched condition.

To determine whether endothelium contributed to the production of these prostanoids, a separate series of experiments were undertaken in which the endothelium was mechanically removed. The stretch failed to augment the steady state releases of thromboxane A_2 (basal, 2.21 ± 0.44 pg/min/mg wet tissue; after stretch, 2.80 ± 0.24 pg/min/mg wet tissue, each $n = 4$), prostaglandin $F_{2\alpha}$ (basal, 8.70 ± 0.77 pg/min/mg wet tissue; after stretch, 5.87 ± 0.67 pg/min/mg wet tissue, each $n = 4$), prostaglandin E_2 (basal, 5.44 ± 1.10

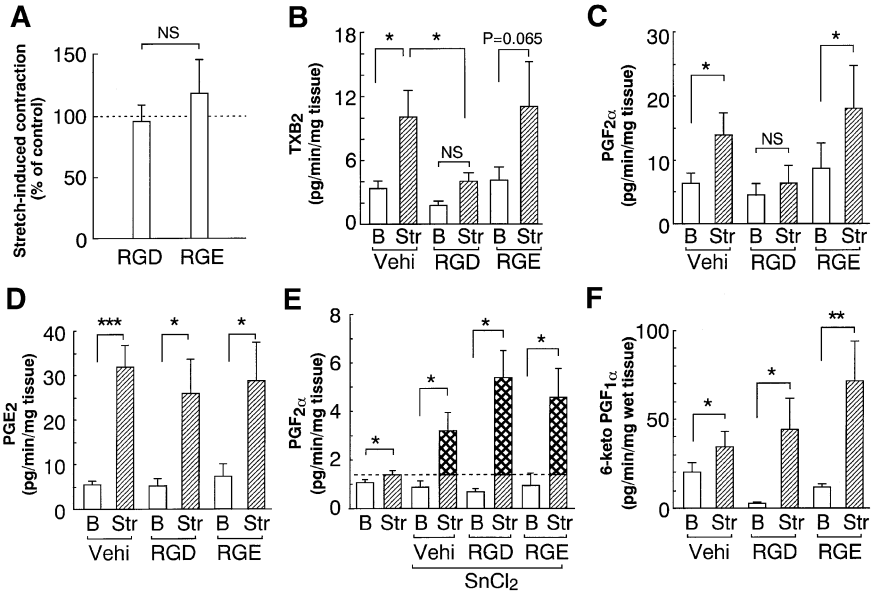

Fig. 3. Effects of RGD peptides on the stretch-induced production of various prostanoids in rabbit pulmonary arteries. (A) Summarized results represent the effects of RGD peptide (left column), an active peptide or RGE peptide (right column), an inactive one, on the stretch-induced contraction of the pulmonary arteries. The artery was pretreated with 100 μM GRGDSP (RGD peptide) or GRGESP (RGE peptide) for 30 min prior to the stretch. Data are presented as a percent of control contraction (=100%), and each column with bar represents the mean ± S.E.M. of 8 (RGD) or 10 (RGE) independent arteries. NS: not statistically significant. (B–F) Summarized results represent the effects of RGD peptide, an active peptide or RGE peptide, an inactive one, or vehicle (Vehi) on the stretch-induced production of various prostanoids in the pulmonary artery. Arteries having intact endothelium were pretreated with either RGD or RGE peptides (each 100 μM) or vehicle for 30 min. Then, the amounts of thromboxane A_2 (B, as TXB_2), prostaglandin $F_{2\alpha}$ (C), prostaglandin E_2 (D), untransformed prostaglandin H_2 (E, cross-hatched area in the columns), and prostaglandin I_2 (F, as 6-keto $PGF_{1\alpha}$) in the bathing solution were measured before (open columns) and after (hatched columns) the stretch as described in the caption of Fig. 2. Each column with bar represents the mean ± S.E.M. of the following numbers of independent arteries; thromboxane A_2: vehicle ($n = 16$), RGD peptide ($n = 5$), RGE peptide ($n = 11$); prostaglandin $F_{2\alpha}$: vehicle ($n = 11$), RGD peptide ($n = 6$), RGE peptide ($n = 5$); PGE_2: vehicle ($n = 10$), RGD peptide ($n = 6$), RGE peptide ($n = 4$); untransformed prostaglandin H_2: vehicle ($n = 6$), RGD peptide ($n = 4$), RGE peptide ($n = 4$); prostaglandin I_2: vehicle ($n = 4$), RGD peptide ($n = 5$), RGE peptide ($n = 5$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, as indicated. B: basal release, Str: stretch.

pg/min/mg wet tissue; after stretch, 3.09 ± 0.52 pg/min/mg wet tissue, each $n=4$), and untransformed prostaglandin H_2 (basal, 0.50 ± 0.15 pg/min/mg wet tissue; after stretch, 0.39 ± 0.32, each $n=4$) in the arteries (Fig. 2A and B, (−) Endothelium). These results suggest that the stretch-induced production of prostanoids including untransformed prostaglandin H_2 is mostly dependent on endothelium.

It should be noted that the basal level of prostaglandin $F_{2\alpha}$ is greater in endothelium-denuded arteries than that in controls. Thus, it could be an alternative possibility that endothelial layer acts on the basal release of prostaglandin $F_{2\alpha}$ in a negative fashion. Nonetheless, no appreciable augmentation of the production of prostaglandin $F_{2\alpha}$ was observed in the arteries without endothelium in response to the stretch.

3.2. Effects of RGD peptides on mechanical responses and productions of prostanoids to stretch in rabbit pulmonary artery

In order to clarify whether the interaction of integrin with extracellular matrix was involved in the mechanism of stretch-induced contraction of pulmonary artery, we tested the effect of RGD peptide, a blocker of interaction of integrin with extracellular matrix (Pierschbacher and Ruoslahti, 1987), and RGE peptide, an inactive analogue, on the contraction. Both RGD and RGE peptides (each 100 μM) did not affect the stretch-induced contraction (control = 100%) of the rabbit pulmonary artery with intact endothelium (Fig. 3A).

RGD peptide (100 μM), which had no apparent effect on the basal release of prostanoids, significantly inhibited the stretch-induced productions of thromboxane A_2 (Fig. 3B, $n=5$, $P<0.05$), and strongly attenuated the augmentation of production of prostaglandin $F_{2\alpha}$ in response to stretch (Fig. 3C). RGE peptide (100 μM) showed no apparent effect on both productions of thromboxane A_2 and prostaglandin $F_{2\alpha}$ (Fig. 3B and C). In contrast, both RGD and RGE peptides (each 100 μM), did not affect the stretch-induced productions of prostaglandin E_2, untransformed prostaglandin H_2, and prostaglandin I_2 (Fig. 3D, E, and F).

These results suggest that the interaction of integrin with extracellular matrix, which is sensitive to RGD peptide, is involved in the stretch-induced productions of thromboxane A_2 and prostaglandin $F_{2\alpha}$, whereas the interaction mechanism plays a minor role in those of prostaglandin E_2, untransformed prostaglandin H_2, and prostaglandin I_2.

3.3. Effects of ozagrel on the contraction and production of thromboxane A_2 produced by stretch in rabbit pulmonary artery

We previously reported that ozagrel (10 μM), an inhibitor of thromboxane synthase (Uchida and Murao, 1981), inhibited both production of thromboxane A_2 and contraction of rabbit pulmonary artery with intact endothelium in response to stretch (Nakayama et al., 1997). Thus we had considered that thromboxane A_2 was responsible for the stretch-induced contraction of rabbit pulmonary artery. However, ozagrel inhibits thromboxane A_2 biosynthesis in various animal species, such as human, rabbit, dog and guinea pig with IC_{50} (50% inhibitory concentration) values ranging from 10 nM to 1 μM (Hiraku et al., 1986). Thus, the inhibitor at a high concentration of 10 μM may block the production of other prostanoids, including prostaglandin $F_{2\alpha}$. Furthermore, the contraction was produced without augmentation of production of thromboxane A_2 in response to stretch (see Fig. 3A and B). Therefore, we reexamined the effect of ozagrel at a low concentration (10 nM) on the stretch-induced production of thromboxane A_2 and contraction.

Ozagrel (10 nM) abolished the production of thromboxane A_2 in response to stretch (Fig. 4B) without any apparent effect on the stretch-induced contraction (Fig. 4A) and production of prostaglandin $F_{2\alpha}$ (Fig. 4C), suggesting a minor role of thromboxane A_2 for the stretch-induced contraction of rabbit pulmonary artery.

3.4. Prostaglandin H_2 induces contraction of rabbit pulmonary artery

It seems possible that the productions of untransformed prostaglandin H_2 and/or prostaglandin E_2, both of which are

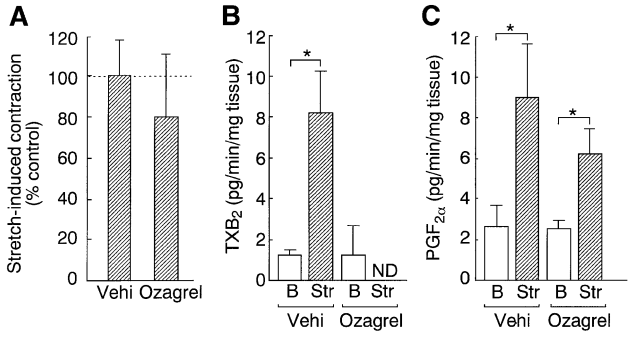

Fig. 4. Effects of ozagrel on stretch-induced contraction and production of thromboxane A_2 and prostaglandin $F_{2\alpha}$ in rabbit pulmonary arteries. (A) Summarized results representing the effect of 10 nM ozagrel on the stretch-induced contraction of rabbit pulmonary arteries with intact endothelium. Data are presented as the percent of control contraction (= 100%), and each column with bar represents the mean ± S.E.M. of four independent arteries. The artery was pretreated with 10 nM ozagrel for 30 min prior to the stretch. (B) Effect of 10 nM ozagrel on the stretch-induced production of thromboxane A_2. The amount of thromboxane B_2, a stable metabolite of thromboxane A_2, in the bathing solution of vehicle (Vehi)- or ozagrel-treated (Ozagrel) pulmonary arteries with intact endothelium was measured before (open columns) or after (hatched columns) stretch. Each column with bar represents the mean ± S.E.M. of five independent arteries. *$P<0.05$, as indicated. B: basal release, Str: stretch, ND: not detectable. (C) Effect of 10 nM ozagrel on the stretch-induced production of prostaglandin $F_{2\alpha}$. The amount of prostaglandin $F_{2\alpha}$ in the same sample as in (B) was measured.

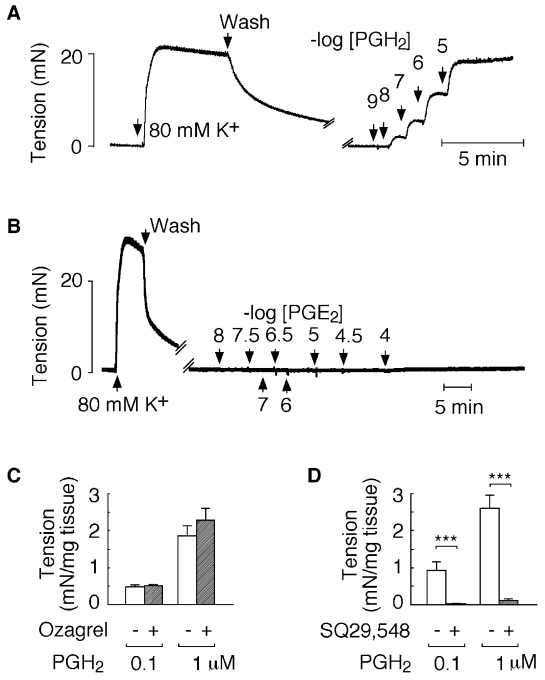

Fig. 5. Effects of exogenous prostaglandin E_2 or prostaglandin H_2 on the contraction of rabbit pulmonary arteries without endothelium. (A) Typical tracings show the effects 80 mM KCl and exogenous prostaglandin H_2 on the rabbit pulmonary artery without endothelium. Prostaglandin H_2 was cumulatively applied to the pulmonary artery ring at an optimum passive load of about 10 mN. (B) Typical tracings show the effects of 80 mM KCl and exogenous prostaglandin E_2 on the rabbit pulmonary artery without endothelium. Prostaglandin E_2 was cumulatively applied to the pulmonary artery ring at an optimum passive load of about 10 mN. (C) Contractile effects of exogenous prostaglandin H_2 on the endothelium-removed pulmonary artery. Prostaglandin H_2 was applied to the arteries in the presence (hatched column) or absence (open column) of 10 μM ozagrel. Ozagrel was added 30 min before administration of prostaglandin H_2. Each column with bar represents the mean ± S.E.M. of four independent arteries. (D) Effect of 100 nM SQ29,548, a TP-receptor antagonist, on the exogenous prostaglandin H_2-induced contraction of the endothelium-denuded pulmonary artery. Prostaglandin H_2 at indicated concentrations was applied to the denuded artery in the presence (hatched column) or absence (open column) of 100 nM SQ29,548. The antagonist was added 5 min before administration of prostaglandin H_2. Each column with bar represents the mean ± S.E.M. of 4–8 independent arteries. ***$P<0.001$, as indicated.

insensitive to RGD peptide, are responsible for the stretch-induced contraction of the rabbit pulmonary artery, because both of them have been reported to be constrictive in human (Qian et al., 1994) and rat pulmonary arteries (Jourdan et al., 1997). Therefore, we examined the effects of prostaglandin H_2 or prostaglandin E_2 on endothelium-removed rabbit pulmonary arteries. Exogenous prostaglandin H_2 in a range between 1 nM and 10 μM contracted the pulmonary artery in a concentration-dependent fashion with EC_{50} value of about 500 nM (Fig. 5A). The tension induced by the prostaglandin H_2 (10 μM) was about 6 mN/mg wet tissue, and it was comparable with that produced by 80 mM KCl (Fig. 5A). In contrast, prostaglandin E_2, added cumulatively to the bathing medium, showed no significant contraction of the artery (Fig. 5B). Though it was reported that prostaglandin E_2 acted dilative on the preconstricted pulmonary vessels of fetal and early newborn lambs (Barnes and Liu, 1995), prostaglandin E_2 did not show any dilatory effect on the endothelium-removed pulmonary artery precontracted with prostaglandin H_2 (data not shown).

The contraction of rabbit pulmonary artery produced by exogenous prostaglandin H_2 was not appreciably affected in the presence of excessive concentration of ozagrel (10 μM) (Fig. 5C), which could rule out a possibility of molecular conversion of exogenous prostaglandin H_2 into thromboxane A_2 by endogenous thromboxane synthase in components including smooth muscles and adventitia. To the contrary, the contraction of the endothelium-removed pulmonary artery by prostaglandin H_2 was effectively inhibited

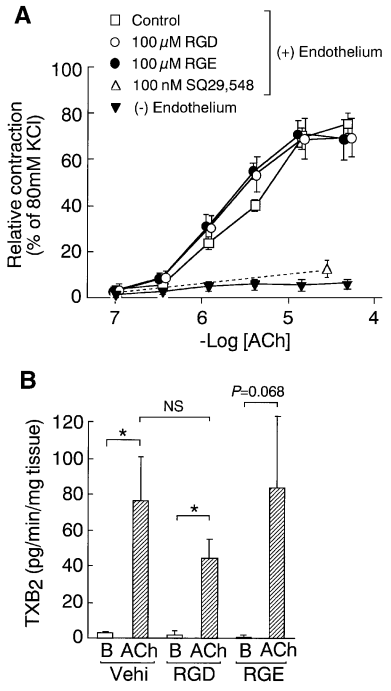

Fig. 6. Effects of RGD peptides on contraction and thromboxane A_2-production by rabbit pulmonary artery in response to acetylcholine. (A) Concentration–response curves for acetylcholine-induced contraction of rabbit pulmonary arteries with or without endothelium in the presence of L-NNA (10 μM). The contraction of the arteries was produced by cumulative administration of acetylcholine in the presence or absence RGD or RGE peptide (each 100 μM). Either GRGDSP (100 μM) or GRGESP (100 μM) was added 30 min before cumulative administration of acetylcholine. Abolition of acetylcholine-induced contraction of the arteries by SQ29,548 was also indicated. Data are presented as a percent of maximum contraction of each artery induced by 80 mM KCl (=100%), and each point with bar represents the mean ± S.E.M. of seven independent arteries. ACh: acetylcholine. (B) Effects of RGD and RGE peptides on acetylcholine-induced production of thromboxane A_2. The amount of thromboxane B_2, a stable metabolite of thromboxane A_2, in the bathing solution of vehicle (Vehi)-, RGD-, and RGE-treated pulmonary arteries were measured before (open columns) and after (hatched columns) addition of 30 μM acetylcholine. Each column with bar represents the mean ± S.E.M. of the following numbers of independent arteries: vehicle ($n=12$), RGD peptide ($n=7$), and RGE peptide ($n=5$). *$P<0.05$, as indicated. B: basal release. ACh: acetylcholine.

by SQ29,548 (100 nM) (Fig. 5D), a competitive antagonist of TP-receptor, being consistent with the susceptibility of stretch-induced contraction to this antagonist.

3.5. Effects of RGD peptide and ozagrel on contraction and production of thromboxane A_2 in response to acetylcholine in rabbit pulmonary artery

Acetylcholine (0.1–30 μM) produced contraction of only the pulmonary artery with intact endothelium in a concentration-dependent manner (Fig. 6A). In addition, effective blockade of the acetylcholine-induced contraction by SQ29,548 (Fig. 6A) reconfirmed the involvement of TP-receptor pathway in the endothelium-dependent contraction of rabbit pulmonary artery (Nakayama et al., 1997). Both RGD and RGE peptides (each 100 μM) showed no apparent effect on the maximum contraction amplitude (control, 75.8 ± 4.5%, $n = 14$; +RGD, 71.1 ± 5.5%, $n = 7$; +RGE, 69.2 ± 8.5%, $n = 7$; 80 mM KCl = 100%). EC_{50} values in the presence of each peptide slightly but significantly decreased as compared with the control value (+RGD, 2.02 ± 0.39 μM; +RGE, 2.26 ± 0.40 μM; each $n = 7$, $P < 0.05$ vs. control, 4.42 ± 0.65 μM, $n = 14$). No statistical difference, however, was observed between the values with RGD and RGE peptides. Thus, these results suggest that RGD peptide-sensitive pathway(s) is not involved in acetylcholine-induced contraction of rabbit pulmonary artery with intact endothelium.

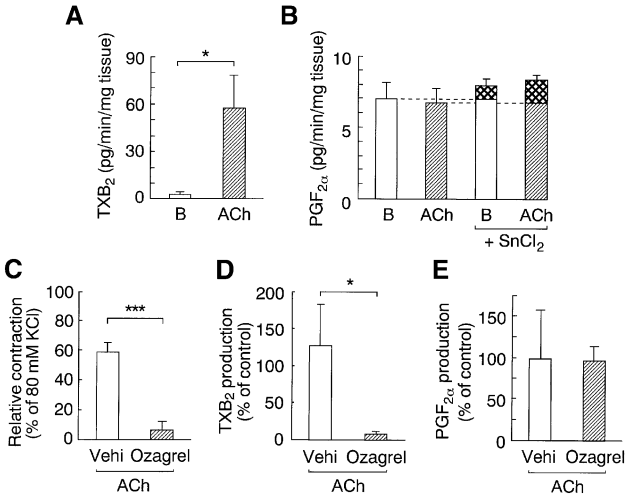

Fig. 7. Effects of ozagrel on acetylcholine-induced production of thromboxane A_2 and contraction. (A) Thromboxane B_2, a stable metabolite of thromboxane A_2, in the bathing solution of the pulmonary artery with intact endothelium was measured before (open column) and after (hatched columns) application of 30 μM acetylcholine. Data are presented as the mean ± S.E.M. of eight independent arteries. *$P < 0.05$, as indicated. B: basal release. ACh: acetylcholine. (B) Prostaglandin $F_{2\alpha}$ in the bathing solution was measured before (open columns) and after (hatched columns) application of 30 μM acetylcholine. Untransformed prostaglandin H_2 (cross-hatched area in each column) was estimated as described in the caption of Fig. 3. Data are indicated as the mean ± S.E.M. of four independent arteries. B: basal release. ACh: acetylcholine. (C) Summarized results represent the effect of 1 μM ozagrel on the 30 μM acetylcholine-induced contraction of the rabbit pulmonary arteries. Data are presented as a percent of control contraction (80 mM KCl = 100%) in the presence (Ozagrel, hatched column) or absence (Vehi, open column) of 1 μM ozagrel. The artery pretreated with 1 μM ozagrel or vehicle for 30 min was challenged by 30 μM acetylcholine. Each column with bar represents the mean ± S.E.M. of four independent arteries. ***$P < 0.001$, as indicated. (D) Effect of 1 μM ozagrel on the acetylcholine-induced production of thromboxane A_2. The amount of thromboxane B_2 was measured in the bathing solution of pulmonary arteries treated or untreated with ozagrel. Data are presented as a percent of the control production of thromboxane B_2 (= 100%) in the presence (hatched column) or absence (Vehi, open column) of 1 μM ozagrel. Each column with bar represents the mean ± S.E.M. of four independent arteries. *$P < 0.05$, as indicated. (E) Effect of 1 μM ozagrel on the acetylcholine-induced production of prostaglandin $F_{2\alpha}$. The amount of prostaglandin $F_{2\alpha}$ in the same sample as in (D) was measured, and indicated as a percent of the control production of prostaglandin $F_{2\alpha}$ (= 100%) as described in the caption of Fig. 3.

Neither RGD nor RGE peptide (each 100 μM) showed any notable effect on the acetylcholine-induced production of thromboxane A_2 (Fig. 6B), suggesting that the acetylcholine-induced production of thromboxane A_2 in the endothelium of rabbit pulmonary artery was not mediated by RGD peptide-sensitive integrins.

We confirmed that acetylcholine (30 μM) significantly increased the production of thromboxane A_2 in the pulmonary artery with intact endothelium (Fig. 7A). However, neither prostaglandin $F_{2\alpha}$ nor untrasformed prostaglandin H_2 was produced in response to acetylcholine (Fig. 7B).

Ozagrel (1 μM) significantly inhibited both acetylcholine-induced contraction (before, 58.5 ± 3.5%; after ozagrel, 6.5 ± 3.0%; 80 mM KCl = 100%, each $n = 4$, $P < 0.001$, Fig. 7C) and the production of thromboxane A_2 (Fig. 7D) without any notable effect on the production of prostaglandin $F_{2\alpha}$ (Fig. 7E) of rabbit pulmonary arteries. These results indicated that acetylcholine-induced contraction of the rabbit pulmonary artery was predominantly mediated by endothelium-derived thromboxane A_2.

4. Discussion

4.1. Stretch-induced contraction and production of prostanoids

In the present study, we firstly confirmed our previous report that the contraction of rabbit pulmonary artery in response to stretch was produced in an endothelium-dependent manner, and that it was inhibited by SQ29,548 (Fig. 1A and B). Furthermore, we found in the present study that the stretch significantly augmented the endothelium-dependent releases of several vasoactive prostanoids, such as prostaglandin $F_{2\alpha}$, prostaglandin E_2, prostaglandin I_2 (measured as 6-keto prostaglandin $F_{1\alpha}$), thromboxane A_2 (measured as thromboxane B_2), and untransformed prostaglandin H_2 (measured as increased prostaglandin $F_{2\alpha}$ in the presence of $SnCl_2$) (Fig. 2). Therefore, the molecular species of prostanoids produced by the stretch were somehow different from those produced by acetylcholine, where the produc-

tions of thromboxane A_2 (Fig. 7) and prostaglandin I_2 (Nakayama et al., 1997) were dominant.

As to the products of cyclooxygenases, it is well-documented that various chemical and mechanical stimuli release arachidonate by activation of phospholipase A_2 or other acylhydrolases. Then, the cyclooxygenase forms cyclic endoperoxide prostaglandin G_2, and converts prostaglandin H_2. Prostaglandin H_2 is mainly transformed enzymatically into a variety of products, including thromboxane A_2, prostaglandin $F_{2\alpha}$, prostaglandin E_2, and prostaglandin I_2 or prostaglandin D_2. However, a growing body of evidence has provided that 'untransformed' prostaglandin H_2 derived from endothelium acts on blood vessels (Huang et al., 2000; Soler et al., 2000; Maruyama et al., 1999; Asano et al., 1994; Lin et al., 1994; Shimizu et al., 1993; Fu-Xiang et al., 1992). Several prostanoid species including prostaglandin $F_{2\alpha}$ could be produced by not only endothelial cells but also smooth muscles, as well as adventitia of arterial tissues (Lennon and Poyser, 1986). Indeed, the pulmonary arteries without endothelium released spontaneously various prostanoids as revealed in the present study. Nevertheless, none of them increased in response to stretch (Fig. 2, (−) Endothelium). In addition, it has been considered that endothelial cells are a major source of prostanoids in vascular tissues, and the activities of cyclooxygenase was over 20 times higher than those of smooth muscle (DeWitt et al., 1983; Davidge, 2001). As noted in Section 3, basal production of prostaglandin $F_{2\alpha}$ in the pulmonary artery without endothelium apparently larger than the production in the pulmonary artery with intact endothelium (Fig. 2). There is an alternative possibility that endothelium negatively acted on basal production of prostaglandin $F_{2\alpha}$. Nevertheless, stretch-induced augmentation of prostaglandin $F_{2\alpha}$-production was not observed in the arteries without endothelium. Thus, we considered that stretch-induced production of various prostanoids including untransformed prostaglandin H_2 could be attributable to endothelial cells.

On the other hand, cellular origins of other prostanoids, especially those of thromboxane A_2 and prostaglandin E_2 still remain to be clarified. Earlier pharmacological studies implicated that endothelial cells produced thromboxane A_2 (Altiere et al., 1986; Buzzard et al., 1993; Nakayama et al., 1997). More recently, Ermert et al. (2000) showed a lack of thromboxane synthase in rat and human endothelial cells isolated from lung tissues assessed by immunohistochemical and in situ hybridization procedures. Pfister et al. (1998) also showed no expression of thromboxane synthase in rabbit pulmonary endothelial cells. More recently, they suggested that contaminated platelets adhered to the endothelium contributed thromboxane synthesis in primary cultures of human umbilical vein endothelial cells (Pfister et al., 2002). In contrast, Jiménez et al. (1999) reported that cultured endothelial cells derived from bovine aorta could produce thromboxane A_2. With regard to prostaglandin E synthase, there is also a controversy: Endothelial cells from human umbilical and saphenous veins did not express mRNA encoding prostaglandin E synthase (Soler et al., 2000). Gryglewski et al. (2001) reported that mRNA encoding prostaglandin E synthase could be expressed in human umbilical vein endothelial cells by bradykinin. Therefore, further precise experiments, such that purified and cultured endothelial cells are stretched by an in vitro cell-stretch system (Tanabe et al., 2000), will help to solve the complexity of productions of prostanoids induced by stretch in endothelial cells.

4.2. Involvement of RGD peptide-sensitive and -insensitive pathways in stretch-induced productions of prostanoids

At least 24 different isoforms of integrin consists of various combinations of 18 α and 8 β chains have been described (Bouvard et al., 2001), but the major isoforms of integrins expressed in endothelial cells are $\alpha_v\beta_3$, $\alpha_5\beta_1$, $\alpha_3\beta_1$, and $\alpha_2\beta_1$ (Luscinskas and Lawler, 1994; Lampugnani et al., 1991). Of these, $\alpha_v\beta_3$, $\alpha_5\beta_1$, and $\alpha_3\beta_1$ are RGD peptide-sensitive on their binding to extracellular matrix, whereas $\alpha_2\beta_1$ is insensitive to RGD peptide (Hynes, 1992). In the present study, we found that RGD peptide had no apparent effect on the stretch-induced contraction (Fig. 3A). However, the RGD peptide did significantly inhibit the productions of thromboxane A_2 and prostaglandin $F_{2\alpha}$ (Fig. 3B and C) but did not inhibit those of prostaglandin E_2, untransformed prostaglandin H_2, and prostaglandin I_2 in response to stretch (Fig. 3D–F). Therefore, our present findings indicate that RGD peptide-sensitive and -insensitive pathways may be involved in the stretch-induced production of prostanoids in the endothelium of the pulmonary artery.

It is a notion that integrins are expressed as transmembranal molecules, while various synthases, such as thromboxane and prostacyclin synthases, isomerases for the synthesis of prostaglandin E_2 and prostaglandin D_2, and a reductase catalyzing the conversion of prostaglandin H_2 to prostaglandin $F_{2\alpha}$, are there in the nucleus membrane. Although a molecular assembly between integrins and various enzymes for synthesis of prostanoids is not well clarified, Bhattacharya et al. (2001) recently reported that $\alpha_v\beta_3$-integrin, which is RGD peptide-sensitive, was involved in the activation of cytosolic phospholipase A_2 and arachidonate release in bovine pulmonary artery endothelial cells in response to vitronectin. Thus, they suggested that integrins were involved in the mechanisms for the production of prostanoids. Taken together with our findings and those of others, the interaction between RGD peptide-sensitive integrins and cytosolic phospholipase A_2 might be involved in the stretch-induced production of thromboxane A_2 and prostaglandin $F_{2\alpha}$.

Prostaglandin H_2 is a labile intermediary molecule to be transformed into various terminal prostanoids. Therefore, the release of untransformed prostaglandin H_2 can be considered to occur in a specific circumstance. As described above, several groups (Huang et al., 2000; Soler et al.,

2000; Maruyama et al., 1999; Asano et al., 1994; Lin et al., 1994; Shimizu et al., 1993; Fu-Xiang et al., 1992) have reported the production of untransformed prostaglandin H_2 in vascular endothelium. Our preliminary experiments suggest that an inhibitor of secretory phospholipase A_2 attenuated the contractile response to stretch (unpublished observations), implying a possibility that untransformed prostaglandin H_2 may be produced via a secretory phospholipase A_2 pathway. Thus, it seems possible that there is dual pathway as to prostaglandin H_2 production: one is for usual transformation of prostaglandin H_2 to terminal prostanoids, and another is for untransformed prostaglandin H_2. Further study by use of specific inhibitors of either secretory phospholipase A_2 or cytosolic phospholipase A_2 will be necessary to verify this possibility.

4.3. Endothelium-derived prostaglandin H_2 is responsible for stretch-induced contraction

As described above, RGD peptide inhibited the stretch-induced production of thromboxane A_2 and prostaglandin $F_{2\alpha}$ (Fig. 3B and C), but the peptide did not inhibit stretch-induced contraction (Fig. 3A). Moreover, ozagrel, a thromboxane synthase inhibitor, at 10 nM, completely inhibited the stretch-induced production of thromboxane B_2 (Fig. 4B), but it did not inhibit the contraction (Fig. 4A). Therefore, prostanoids other than thromboxane A_2 and prostaglandin $F_{2\alpha}$ might be involved in the stretch-induced contraction. In contrast, stretch-induced productions of prostaglandin E_2, untransformed prostaglandin H_2, and prostaglandin I_2 in the pulmonary arteries were not affected by RGD peptide (Fig. 3D–F), indicating no apparent involvement of RGD peptide-sensitive integrins, including $\alpha_v\beta_3$, $\alpha_5\beta_1$, and $\alpha_3\beta_1$.

The dual effect of prostaglandin E_2, i.e., it acted vasodilatory and vasoconstrictory on pulmonary arteries of dog and lambs, has been reported (Barnes and Liu, 1995). In the present study, exogenous prostaglandin E_2 showed no apparent contractile effect on the rabbit pulmonary artery (Fig. 5B). The exogenously-applied prostaglandin H_2, on the other hand, acted potently vasoconstrictive (Fig. 5A). The contraction produced by exogenous prostaglandin H_2 was not affected by a thromboxane synthase inhibitor, ozagrel at an excessive concentration of 10 μM (Fig. 5C), but was abolished by a TP-receptor antagonist, SQ29,548 (100 nM) (Fig. 5D), suggesting that prostaglandin H_2 could act directly on the pulmonary artery through TP-receptor. Thus, untransformed prostaglandin H_2 rather than prostaglandin E_2 seems to be responsible for the stretch-induced contraction of the rabbit pulmonary artery.

Acetylcholine-induced contraction of the rabbit pulmonary artery is likely to be attributable to thromboxane A_2, because both contraction and production of thromboxane A_2 were significantly inhibited by ozagrel (1 μM) without any notable effect on the production of prostaglandin $F_{2\alpha}$ (Fig. 7) or prostaglandin I_2 (Nakayama et al., 1997). In addition, we previously showed that the acetylcholine-induced contraction was effectively inhibited by SQ29,548, a TP-receptor antagonist (Nakayama et al., 1997). We confirmed this fact in the present study (Fig. 6A). However, acetylcholine-induced production of thromboxane A_2 in the endothelium was not inhibited by RGD peptide, suggesting that RGD peptide-sensitive integrin pathway plays a minor role in the production of thromboxane A_2 in response to acetylcholine.

As a summary, stretch augments production of prostanoids in the rabbit pulmonary artery endothelium via either RGD peptide-sensitive integrins or RGD-insensitive mechanisms. Untransformed prostaglandin H_2 seems to be a mediator for the stretch-induced contraction of the rabbit pulmonary artery, whereas thromboxane A_2 seems to be causal in acetylcholine-induced contraction.

Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research of the Ministry of Education, Science, Sports, and Culture of Japan (Nos. 08557139, 10672046, and 12470528 to K.N. and Nos. 09772028, 11771443, and 14572059 to Y.T.). The authors thank Drs. Ishikawa, T. and Obara, K. for their valuable discussions.

References

Altiere, R.J., Kiritsy-Roy, J.A., Catravas, J.D., 1986. Acetylcholine-induced contractions in isolated rabbit pulmonary arteries: role of thromboxane A_2. J. Pharmacol. Exp. Ther. 236, 541535.

Asano, H., Shimizu, K., Muramatsu, M., Iwama, Y., Toki, Y., Miyazaki, Y., Okumura, K., Hashimoto, H., Ito, T., 1994. Prostaglandin H_2 as an endothelium-derived contracting factor modulates endothelin-1-induced contraction. J. Hypertens. 12, 383–390.

Barnes, P.J., Liu, S.F., 1995. Regulation of pulmonary vascular tone. Pharmacol. Rev. 47, 87–131.

Bhattacharya, S., Patel, R., Sen, N., Quadri, S., Parthasarathi, K., Bhattacharya, J., 2001. Dual signaling by the $\alpha_V\beta_3$-integrin activates cytosolic PLA_2 in bovine pulmonary artery endothelial cells. Am. J. Physiol.: Lung Cell. Mol. Physiol. 280, L1049–L1056.

Bouvard, D., Brakebusch, C., Gustafsson, E., Aszódi, A., Bengtsson, T., Berna, A., Fässler, R., 2001. Functional consequences of integrin gene mutation in mice. Circ. Res. 89, 211–223.

Buzzard, C.J., Pfister, S.L., Campbell, W.B., 1993. Endothelium-dependent contractions in rabbit pulmonary artery are mediated by thromboxane A_2. Circ. Res. 72, 1023–1034.

Camacho, M., Lopez-Belmonte, J., Vila, L., 1998. Rate of vasoconstrictor prostanoids released by endothelial cells depends on cyclooxygenase-2 expression and prostaglandin I synthase activity. Circ. Res. 83, 353–365.

Davidge, S.T., 2001. Prostaglandin H synthase and vascular function. Circ. Res. 89, 650–660.

DeWitt, D.L., Day, J.S., Sonnenburg, W.K., Smith, W.L., 1983. Concentrations of prostaglandin endoperoxide synthase and prostaglandin I_2 synthase in the endothelium and smooth muscle of bovine aorta. J. Clin. Invest. 72, 1882–1888.

Ermert, L., Ermert, M., Duncker, H.R., Grimminger, F., Seeger, W., 2000. In situ localization and regulation of thromboxane A_2 synthase in nor-

mal and LPS-primed lungs. Am. J. Physiol.: Lung Cell. Mol. Physiol. 278, L744–L753.

Fu-Xiang, D., Skopec, J., Diederich, A., Diederich, D., 1992. Prostaglandin H_2 and thromboxane A_2 are contractile factors in intrarenal arteries of spontaneously hypertensive rats. Hypertension 19, 795–798.

Gryglewski, R.J., Chlopicki, S., Uracz, W., Marcinkiewicz, E., 2001. Significance of endothelial prostacyclin and nitric oxide in peripheral and pulmonary circulation. Med. Sci. Monit. 7, 1–16.

Hamberg, M., Svensson, J., Wakabayashi, T., Samuelsson, B., 1974. Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc. Natl. Acad. Sci. U. S. A. 71, 345–349.

Hiraku, S., Taniguchi, K., Wakitani, K., Omawari, N., Kira, H., Miyamoto, T., Okegawa, T., Kawasaki, A., Ujiie, A., 1986. Pharmacological studies on the TXA_2 synthetase inhibitor (*E*)-3-[*p*-(1*H*-imidazol-1-ylmethyl)phenyl]-2-propenoic acid (OKY-046). Jpn. J. Pharmacol. 41, 393–401.

Huang, A., Sun, D., Koller, A., 2000. Shear stress-induced release of prostaglandin H_2 in arterioles of hypertensive rats. Hypertension 35, 925–930.

Hynes, R.O., 1992. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69, 11–25.

Jiménez, R., Andriambeloson, E., Duarte, J., Andriantsitohaina, R., Jiménez, J., Perez-Vizcaino, F., Zarzuelo, A., Tamargo, J., 1999. Involvement of thromboxane A_2 in the endothelium-dependent contractions induced by myrcetin in rat isolated aorta. Br. J. Pharmacol. 127, 1539–1544.

Jourdan, K.B., Evans, T.W., Curzen, N.P., Mitchell, J.A., 1997. Evidence for a dilator function of 8-iso prostaglandin $F_{2\alpha}$ in rat pulmonary artery. Br. J. Pharmacol. 120, 1280–1285.

Lampugnani, M.G., Resnati, M., Dejana, E., Marchisio, P.C., 1991. The role of integrins in the maintenance of endothelial monolayer integrity. J. Cell Biol. 112, 479–490.

Lennon, E.A., Poyser, N.L., 1986. Production of prostaglandins I_2, E_2 and $F_{2\alpha}$ by blood vessels of normotensive and hypertensive, male and female rats. Prostaglandins Leukotrienes Med. 25, 71–89.

Lin, L., Balazy, M., Pagano, P.J., Nasjletti, A., 1994. Expression of prostaglandin H_2-mediated mechanism of vascular contraction in hypertensive rats: relation to lipoxygenase and prostacyclin synthase activities. Circ. Res. 74, 197–205.

Luscinskas, F.W., Lawler, J., 1994. Integrins as dynamic regulators of vascular function. FASEB J. 8, 929–938.

Maruyama, J., Yokochi, A., Maruyama, K., Nosaka, S., 1999. Acetylcholine-induced endothelium-derived contracting factor in hypoxic pulmonary hypertensive rats. J. Appl. Physiol. 86, 1687–1695.

Masumoto, N., Nakayama, K., Oyabe, A., Uchino, M., Ishii, K., Obara, K., Tanabe, Y., 1997. Specific attenuation of the pressure-induced contraction of rat cerebral artery by herbimycin A. Eur. J. Pharmacol. 330, 55–63.

Nakayama, K., 1982. Calcium-dependent contractile activation of cerebral artery produced by quick stretch. Am. J. Physiol. Heart Circ. Physiol. 242, H760–H768.

Nakayama, K., Tanaka, Y., 1993. Stretch-induced contraction and Ca^{2+} mobilization in vascular tissue. Biol. Signals 2, 241–252.

Nakayama, K., Suzuki, S., Sugi, H., 1986. Physiological and ultrastructural studies on the mechanism of stretch-induced contractile activation in rabbit cerebral artery smooth muscle. Jpn. J. Physiol. 36, 745–760.

Nakayama, K., Tanaka, Y., Fujishima, K., 1989. Potentiation of stretch-induced myogenic tone of cerebral artery by hemolysate and the inhibitory action of calcium antagonists. Eur. J. Pharmacol. 169, 33–42.

Nakayama, K., Ueta, K., Tanaka, Y., Tanabe, Y., Ishii, K., 1997. Stretch-induced contraction of rabbit isolated pulmonary artery and the involvement of endothelium-derived thromboxane A_2. Br. J. Pharmacol. 122, 199–208.

Nakayama, K., Obara, K., Tanabe, Y., Saito, M., Ishikawa, T., Nishizawa, S., 2003. Interactive role of tyrosine kinase, protein kinase C, and Rho/Rho kinase systems in the mechanotransduction of vascular smooth muscles. Biorheology 40, 307–314.

Obara, K., Saito, M., Yamanaka, A., Uchino, M., Nakayama, K., 2001. Involvement of different Ca^{2+} in the rate-dependent stretch-induced contractions of canine basilar artery. Jpn. J. Physiol. 51, 327–335.

Obara, K., Koide, M., Nakayama, K., 2002. 20-Hydroxyeicosatetraenoic acid potentiates stretch-induced contraction of canine basilar artery via PKCα-mediated inhibition of K_{Ca} channel. Br. J. Pharmacol. 137, 1362–1370.

Pfister, S.L., Deinhart, D.D., Campbell, W.B., 1998. Methacholine-induced contraction of rabbit pulmonary artery: role of platelet-endothelial transcellular thromboxane synthesis. Hypertension 31, 206–212.

Pfister, S.L., Hughes, M.J., Rosolowsky, M., Campbell, W.B., 2002. Role of contaminating platelets in thromboxane synthesis in primary cultures of human umbilical vein endothelial cells. Prostaglandins Other Lipid Mediators 70, 39–49.

Pierschbacher, M.D., Ruoslahti, E., 1987. Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity in cell adhesion. J. Biol. Chem. 262, 17294–17298.

Qian, Y.M., Jones, R.L., Chan, K.M., Stock, A.I., Ho, J.K., 1994. Potent contractile actions of prostanoid EP_3 receptor agonists on human isolated pulmonary artery. Br. J. Pharmacol. 113, 369–374.

Shimizu, K., Muramatsu, M., Kakegawa, Y., Asano, H., Toki, Y., Miyazaki, Y., Okumura, K., Hashimoto, H., Ito, T., 1993. Role of prostaglandin H_2 as an endothelium-derived contracting factor in diabetic state. Diabetes 42, 1246–1252.

Soler, M., Camacho, M., Escudero, J.R., Iniguez, M.A., Vila, L., 2000. Human vascular smooth muscle cells but not endothelial cells express prostaglandin E synthase. Circ. Res. 87, 504–507.

Tanabe, Y., Saito, M., Ueno, A., Nakamura, M., Takeishi, K., Nakayama, K., 2000. Mechanical stretch augments PDGF receptor β expression and protein tyrosine phosphorylation in pulmonary artery tissue and smooth muscle cells. Mol. Cell. Biochem. 215, 103–113.

Uchida, Y., Murao, S., 1981. Effects of thromboxane synthetase inhibitors on cyclical reduction of coronary blood flow in dogs. Jpn. Heart J. 22, 971–975.